Metabolomics and its application

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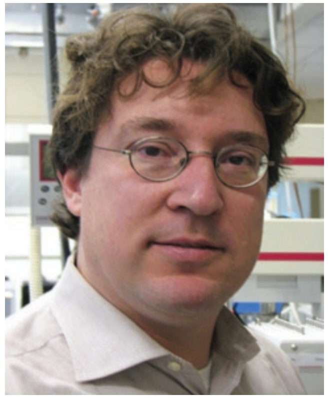
Learning objectives

1. Introduction to Metabolomics
- Metabolomics ?
- Relation to other –omics →systems biology

2. Metabolomics technologies

3. Metabolomics and its application

Metabolomics or Metabonomics ??



"Comprehensive and quantitative analysis of all metabolites…" Fiehn 2001.

"The quantitative measurement of the time-related Multi-parametric metabolic response of living Systems to pathophysiological stimuli or genetic modification" Nicholson et al., 1999

The difference: only philosophy

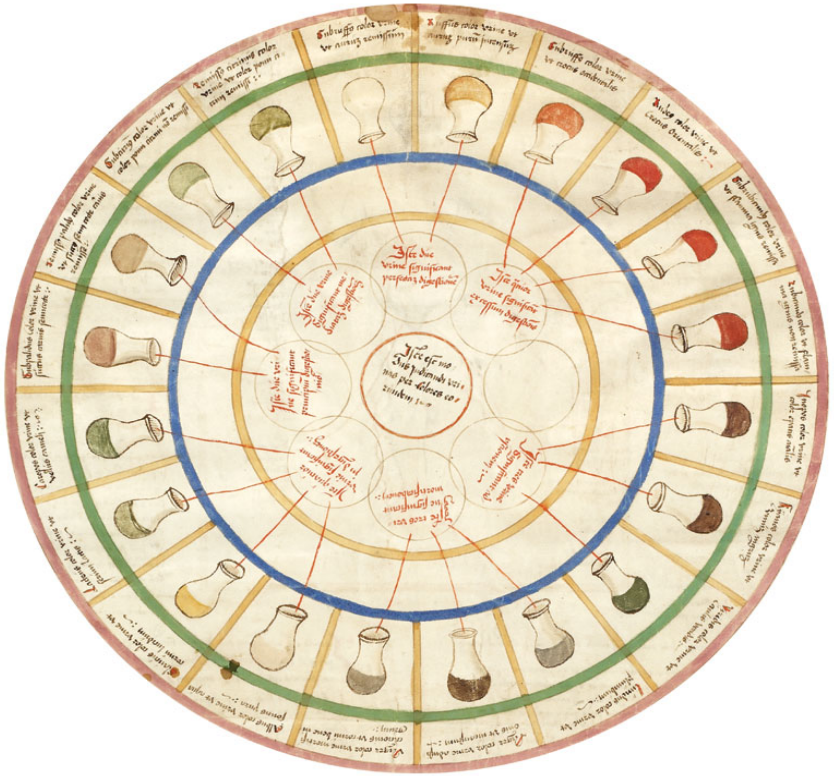
Metabolomics aims to characterize and quantify all small molecules in a sample

Metabonomics aims to measure global dynamic metabolic response of living systems

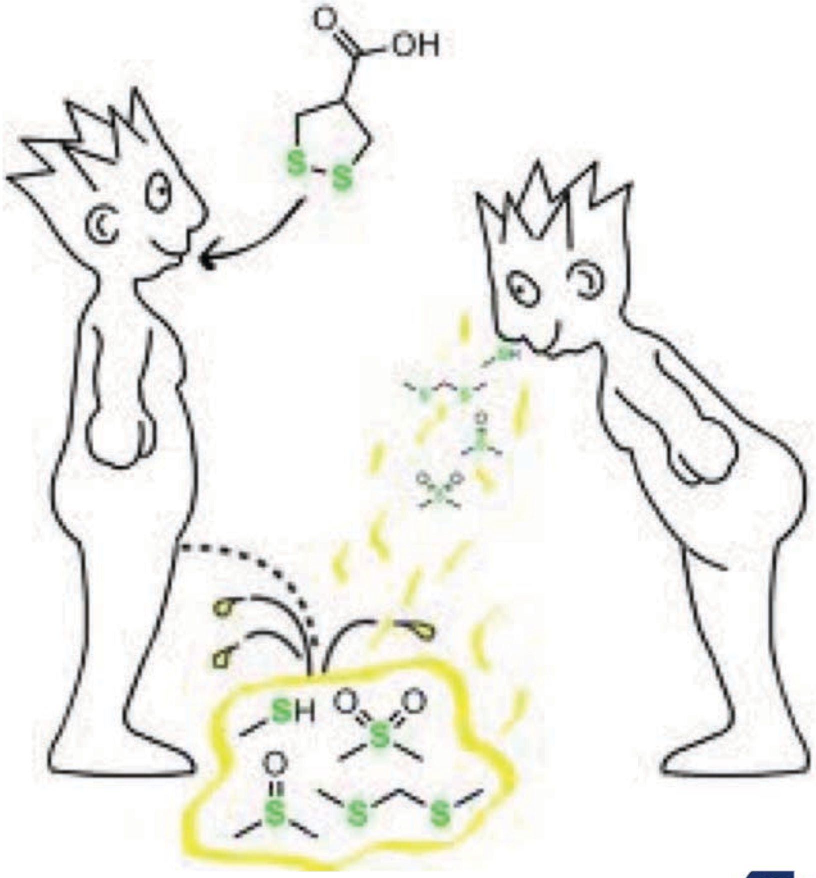
The terms are often used interchangeably, analytical and modeling procedure are the same

Metabolomics in clinical research

The idea that changes in tissues or biological fluid are indicators of disease goes back at least as far back as ancient Greece.



Metabonomics of yore
This urine wheel describes the possible colors, smells, test of urine and uses them to diagnose disease.



Nature (2008) 455 :1054-1056

History and development

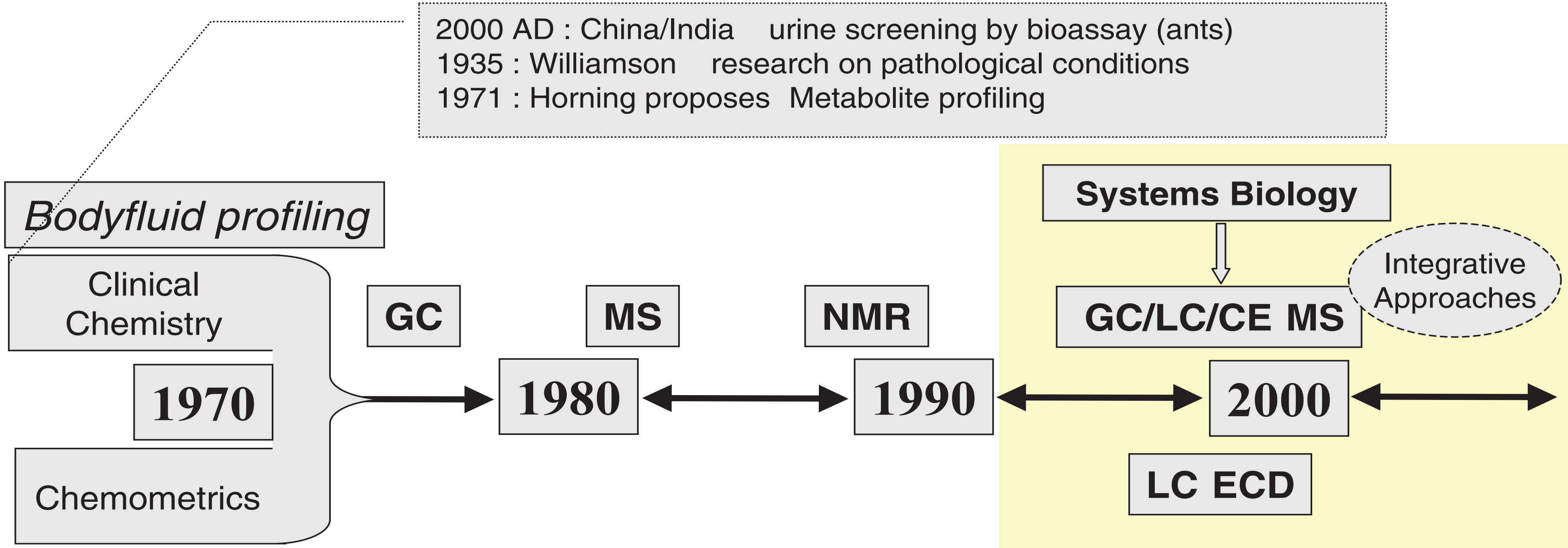


Figure 2. Body fluid profiling. Integration of chemometrics and analytical technologies in biomedical research.

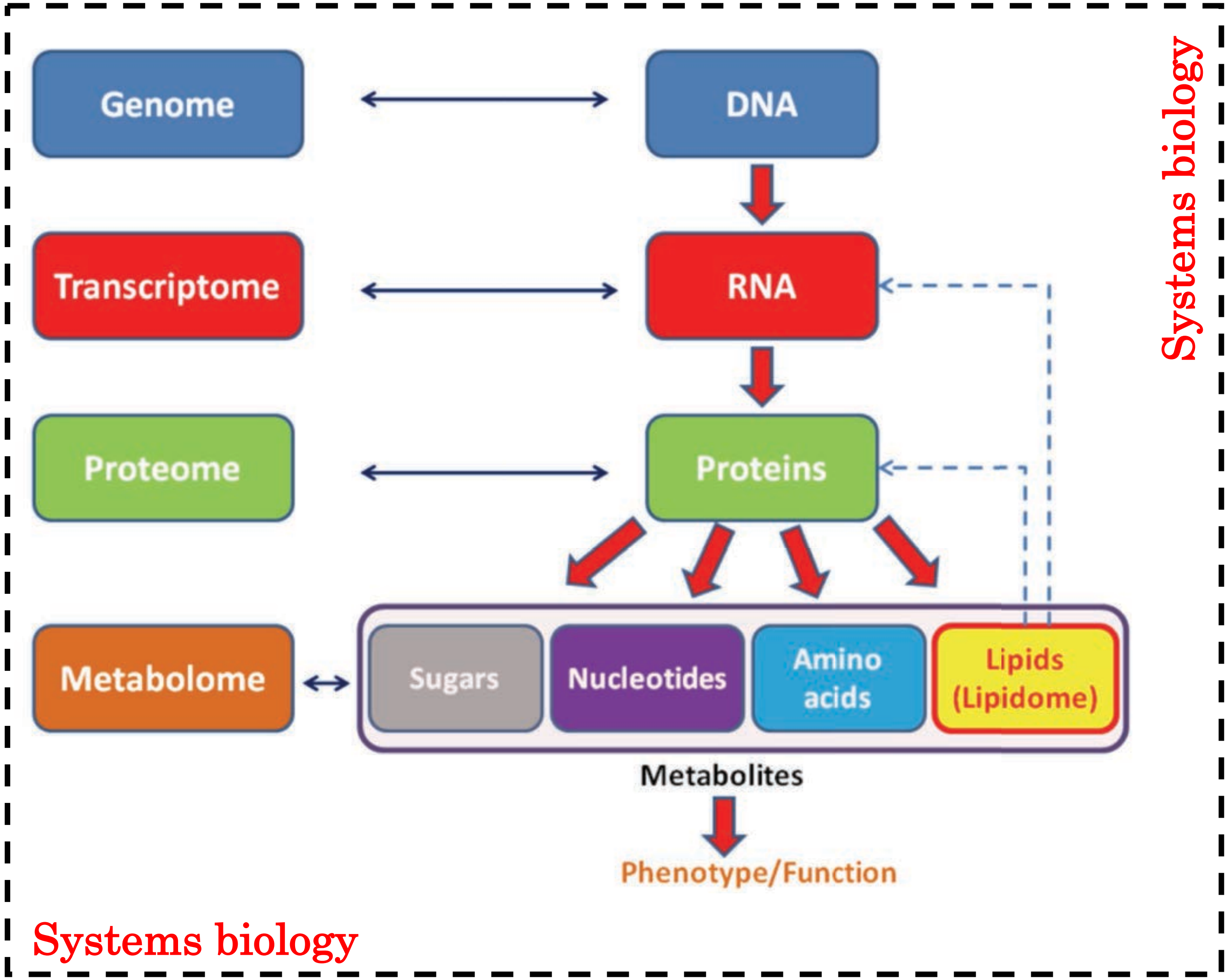
AD = Anno Domini

Metabolomics & other omics

What can happen

What appears to be happening

What has happened and is happening



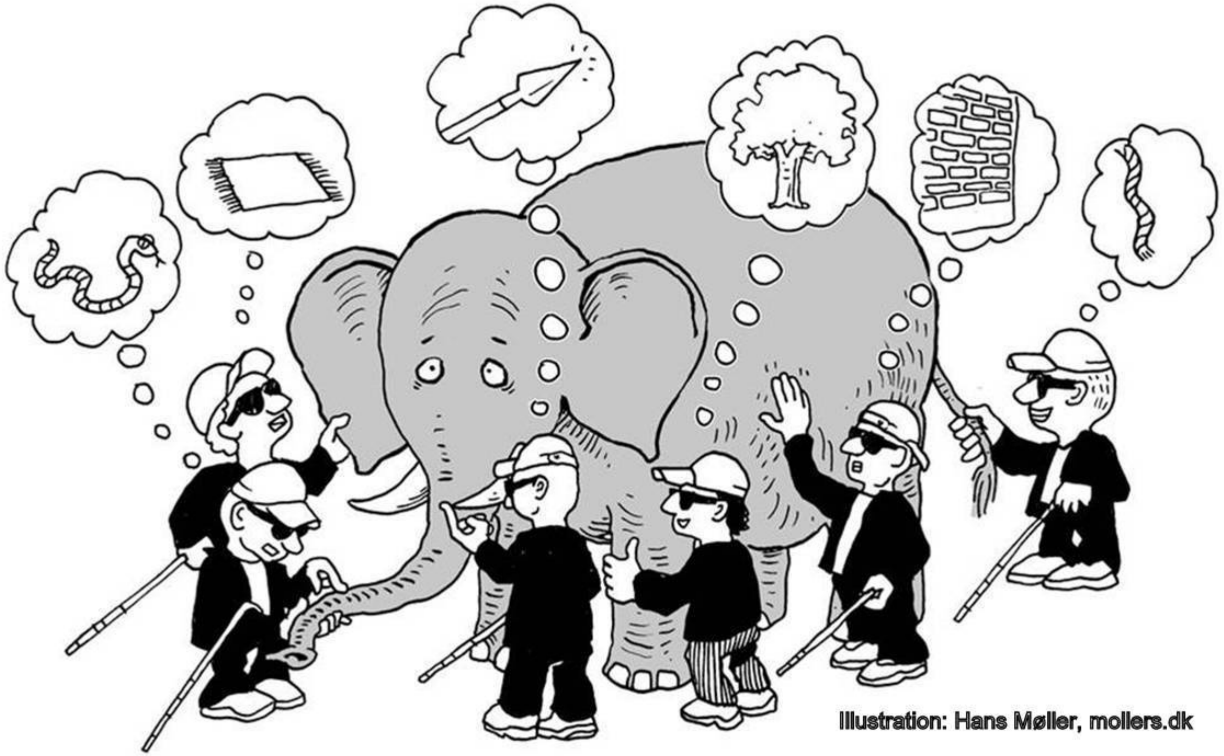
http://en.wikipedia.org/wiki/Lipidomics

Mass spectrometry reviews (2007), 26: 51-78

Systems Biology

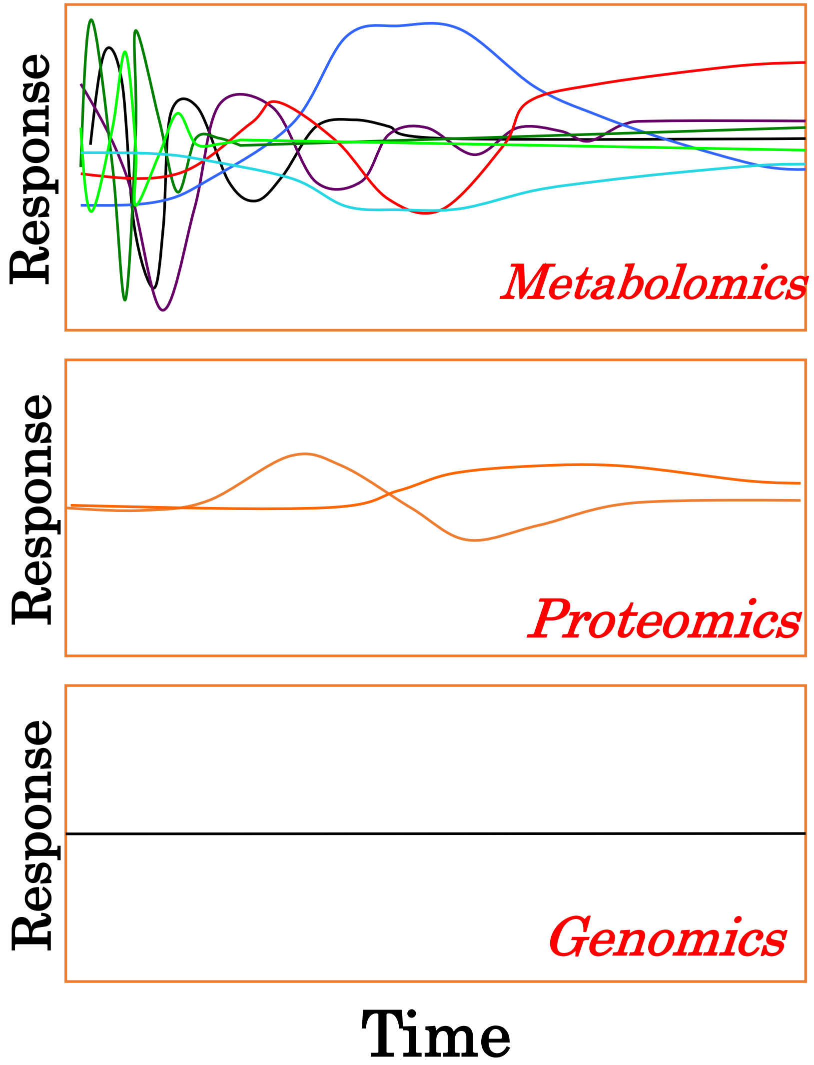
It is a biology-based interdisciplinary field of study that focuses on complex interactions within biological systems, using a holistic approach

"Systems biology...is about putting together rather than taking apart, integration rather than reduction. It requires that we develop ways of thinking about integration that are as rigorous as our reductionist programmes, but different....It means changing our philosophy, in the full sense of the term" (Denis Noble)



The six blind men and the elephant

Metabolomics provides a good sensitivity for quantifying phenotype



Modified from: David Wishart, University of Alberta, Canada

Terms

Metabolites: intermediates and products of metabolism (MW <1k Da) e.g. amino acids, organic acids, sugars, lipids…

Metabolome: a complete set of metabolites in an organism

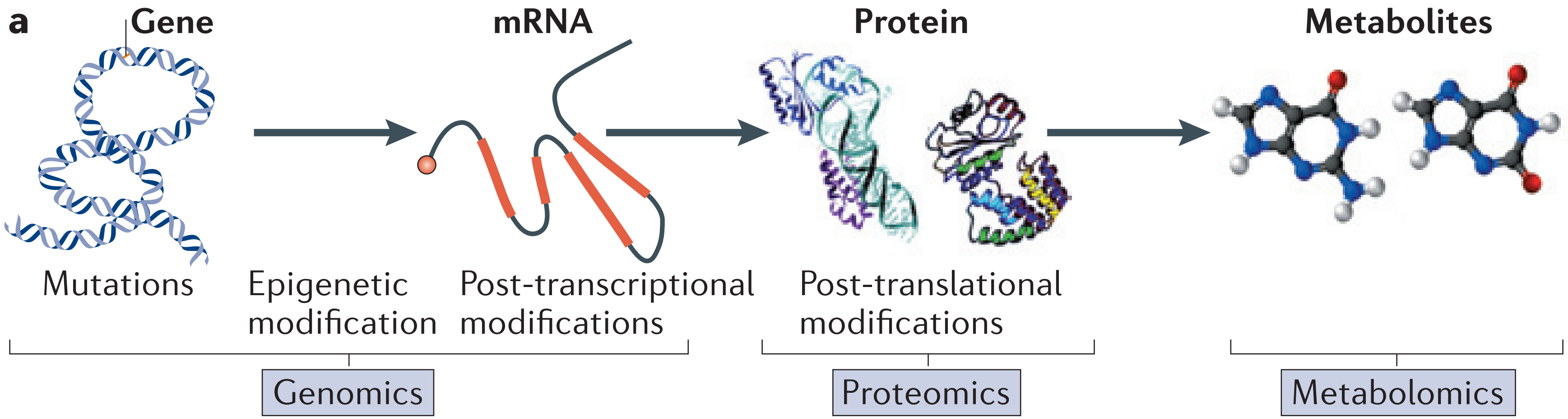
Metabolome is subdivided into endo- and exo-metabolome that cover the intra- and extra- metabolites

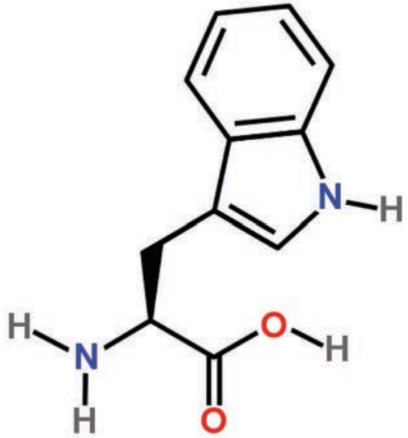
Targeted analysis: only the metabolites of interest

Untargeted analysis or metabolite profiling: as many as possible

What is a metabolite?

- Any organic molecule detectable in the body with a MW < 1500 Da
- Includes peptides, oligonucleotides, sugars, nucelosides, organic acids, ketones, aldehydes, amines, amino acids, lipids, steroids, alkaloids, foods, food additives, toxins, pollutants, drugs and drug metabolites
- Includes human & microbial products
- Concentration > detectable (1 pM)





Metabolites

Plant-based metabolomics:

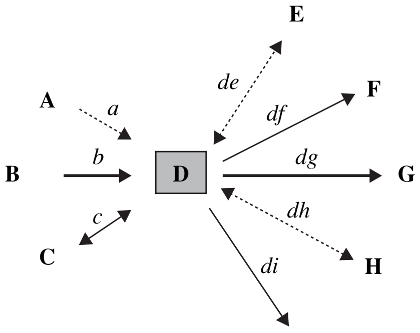
-Primary

-Secondary

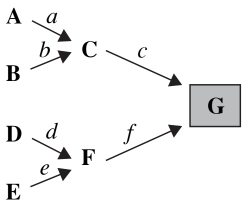
Human-based metabolomics:

-Endogenous

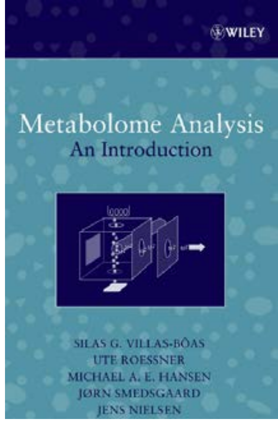
-Exogenous

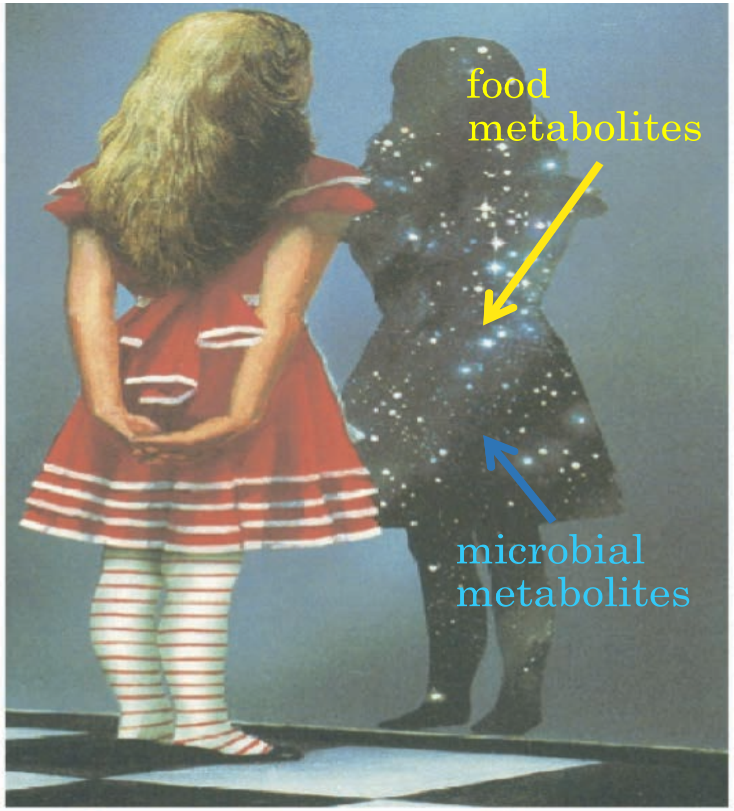


Primary metabolism

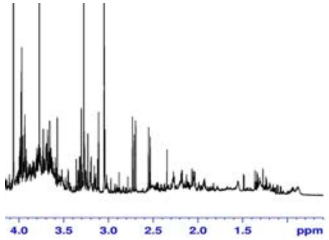


Secondary metabolism

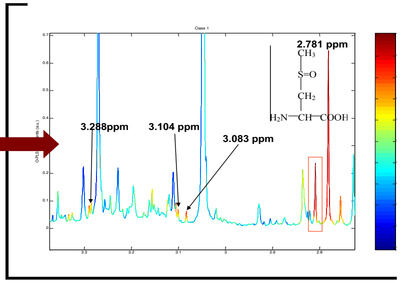




BIOFLUID METABOLITES



DIETARY COMPONENTS



MICROBIAL METABOLITES

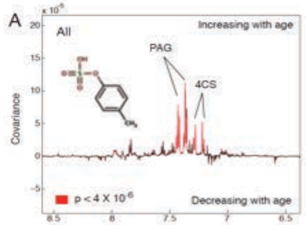
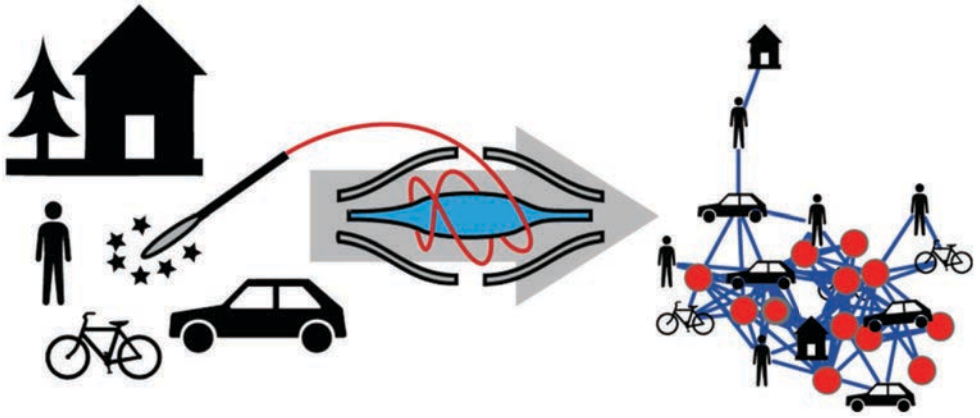
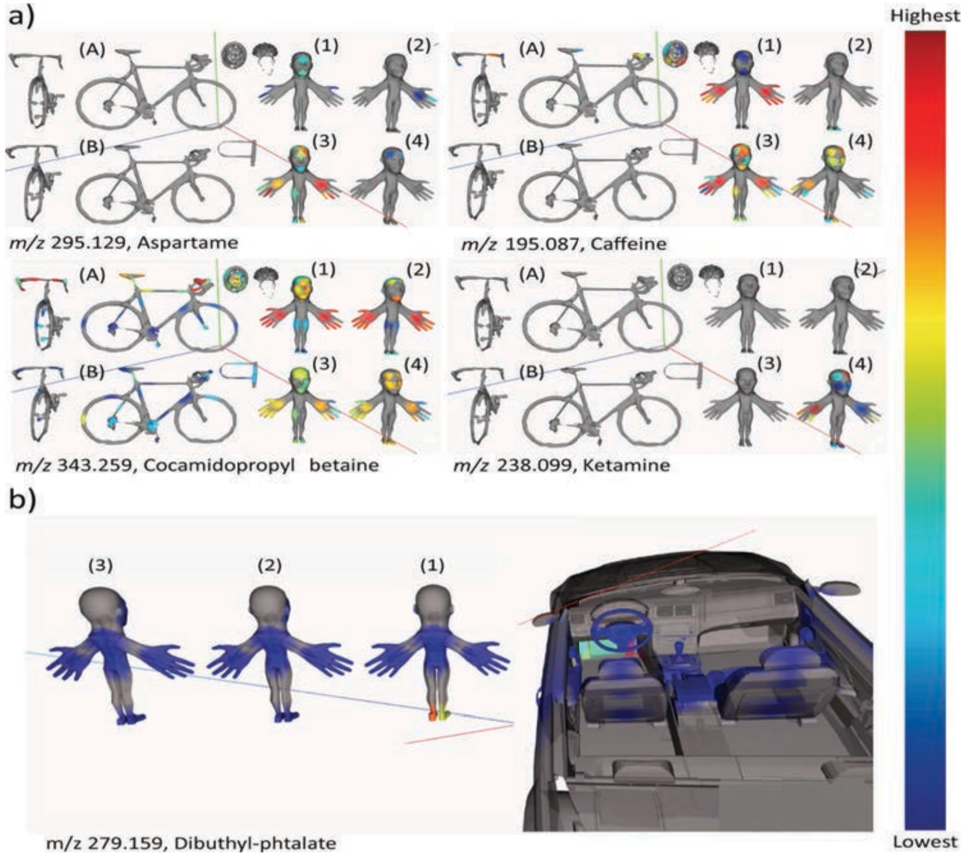
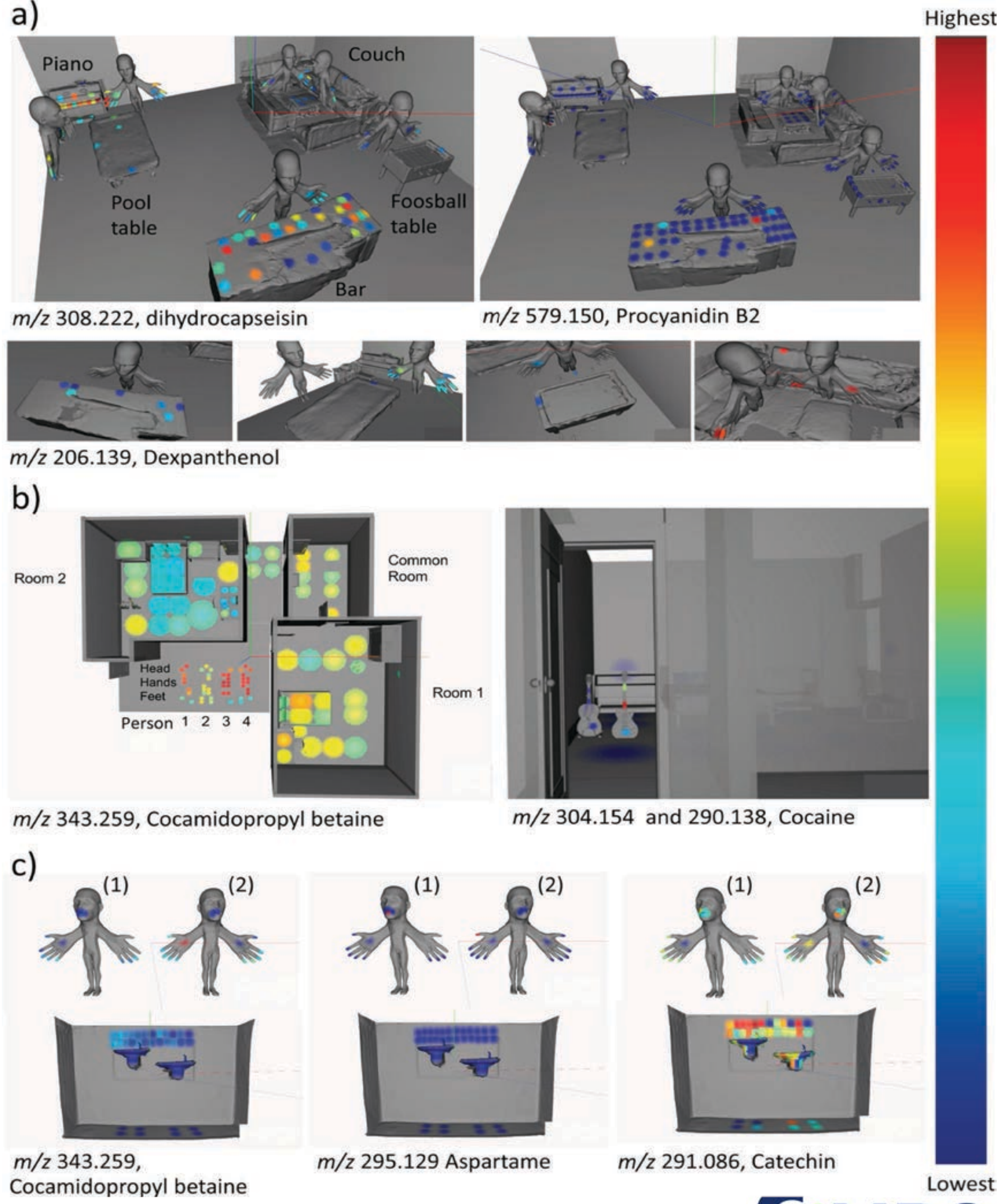


Fig. from: Elanine Holmes, Imperial Collage London



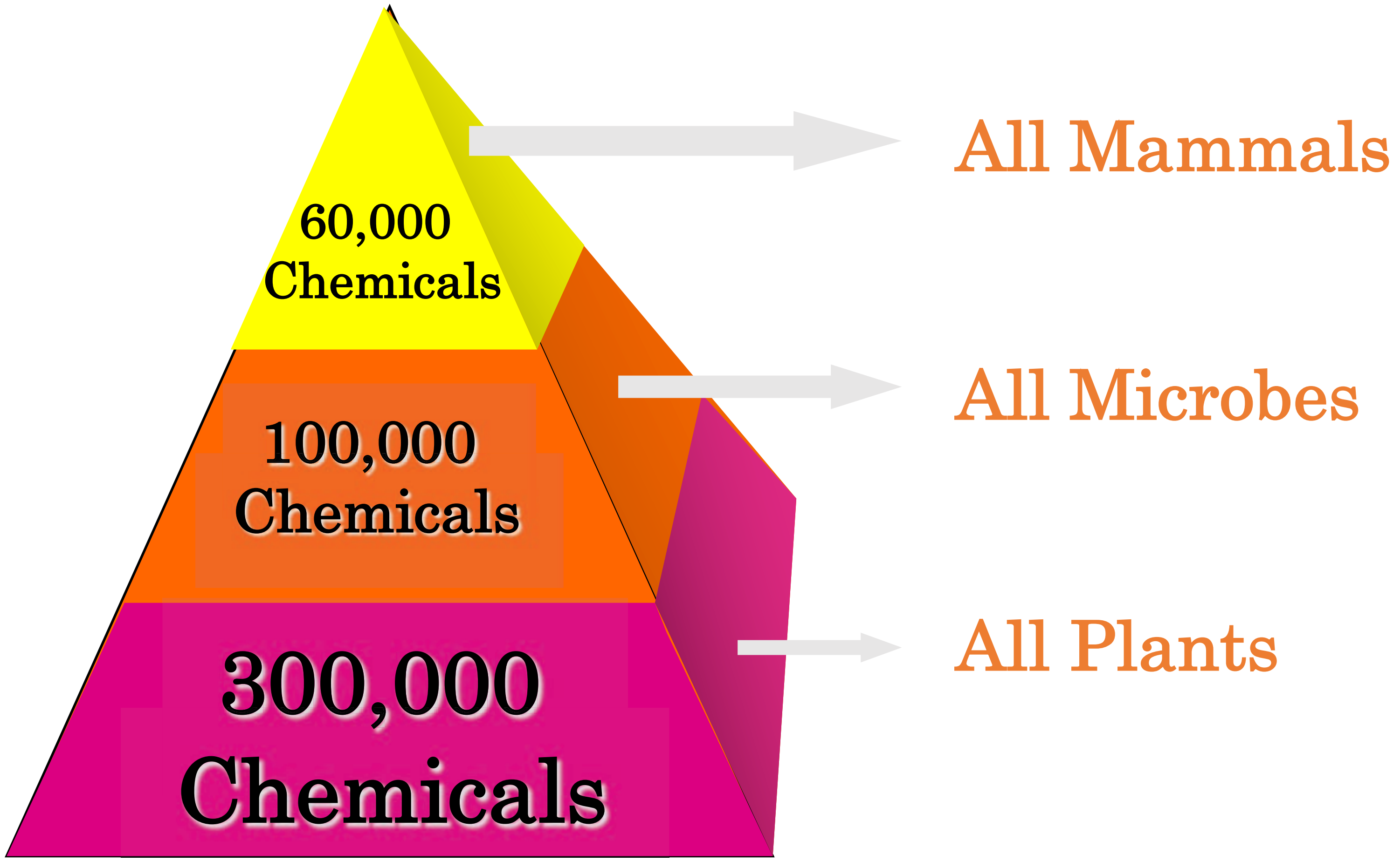


a)
Highest
(A)
(B)
(1)
(2)
(3)
(4)
m/z 295.129, Aspartame
m/z 195.087, Caffeine
m/z 343.259, Cocamidopropyl betaine
m/z 238.099, Ketamine
b)
m/z 279.159, Dibuthyl-phtalate
Lowest



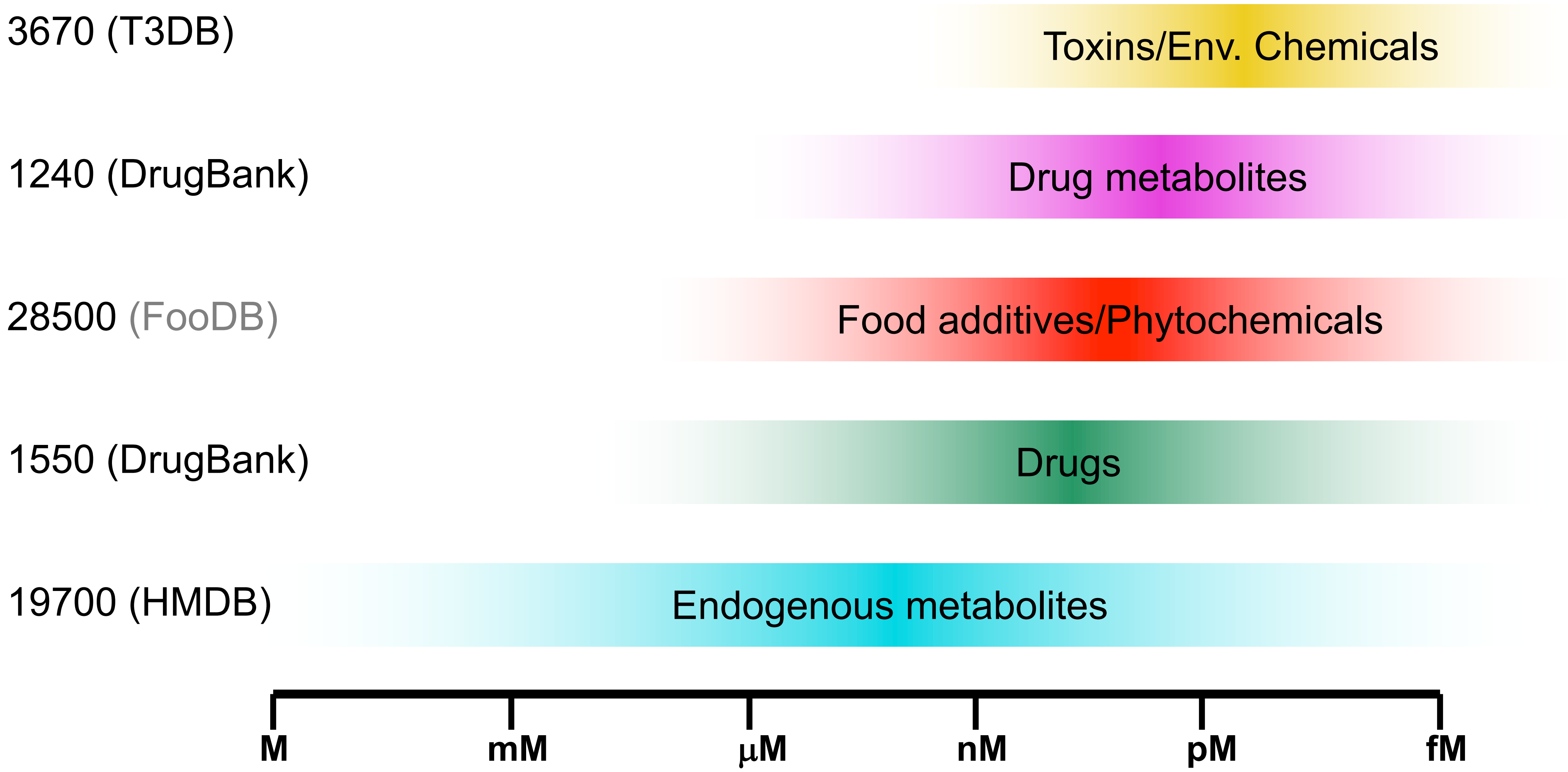
a)
Highest
Piano
Couch
Pool table
Foosball table
Bar
m/z 308.222, dihydrocapseisin
m/z 579.150, Procyanidin B2
m/z 206.139, Dexpanthenol
b)
Room 2
Common Room
Room 1
Head
Hands
Feet
Person 1 2 3 4
m/z 343.259, Cocamidopropyl betaine
m/z 304.154 and 290.138, Cocaine
c)
(1)
(2)
m/z 343.259, Cocamidopropyl betaine
m/z 295.129 Aspartame
m/z 291.086, Catechin
Lowest

Different Metabolomes



The Pyramid of Life

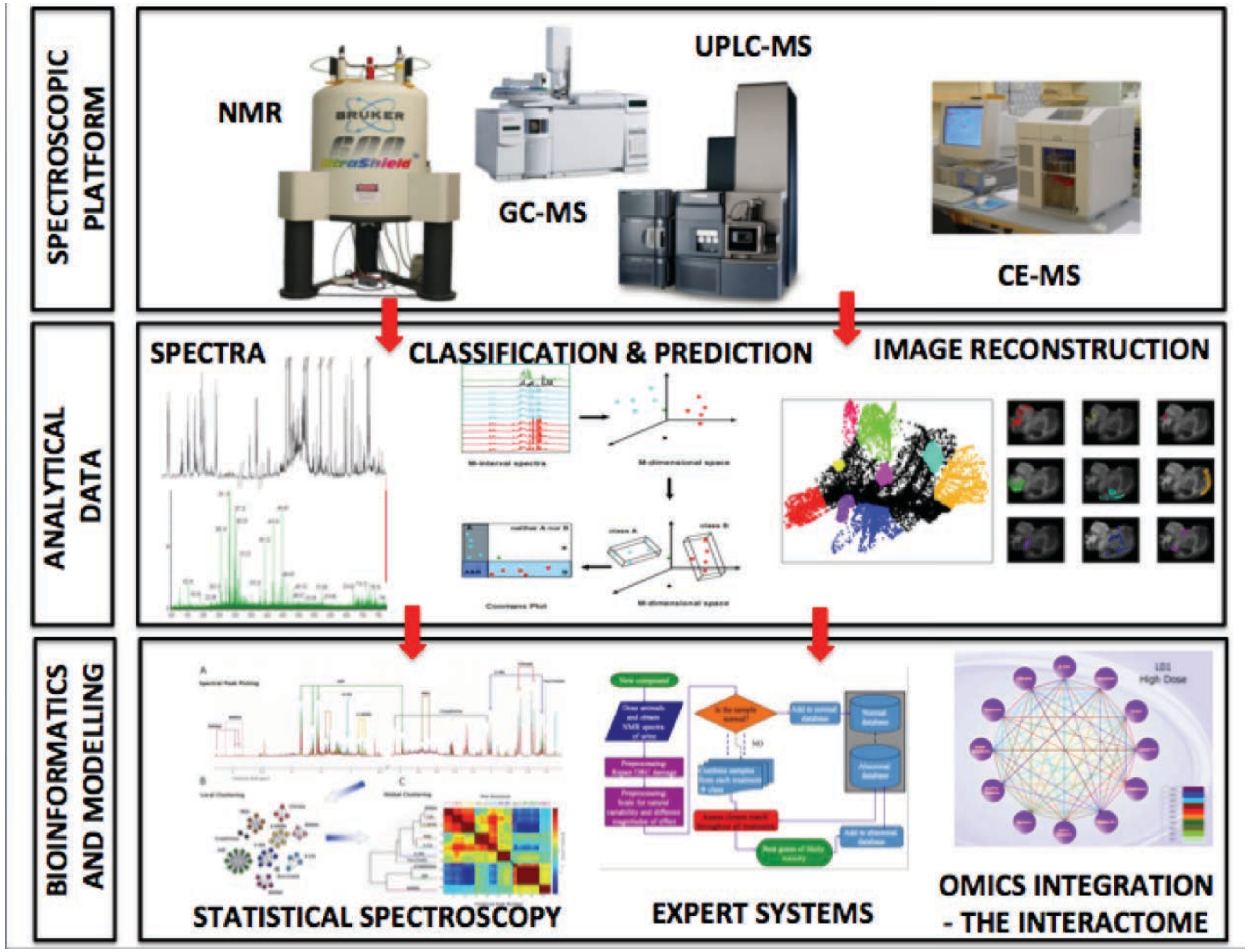
Human Metabolomes (2015)

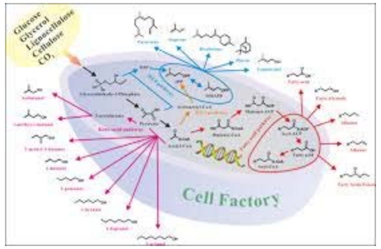


Metabolomics research

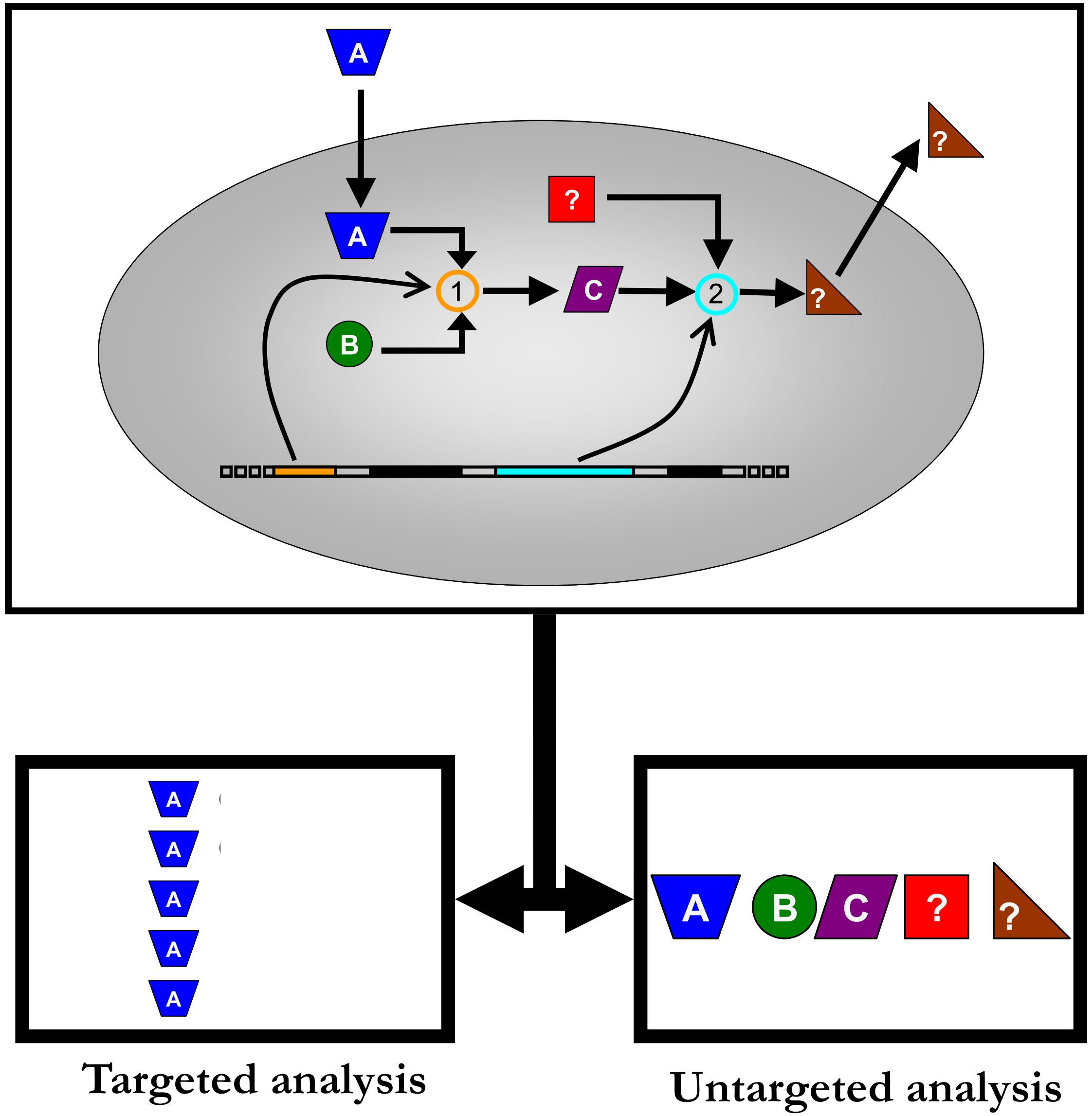
Wet chemistry

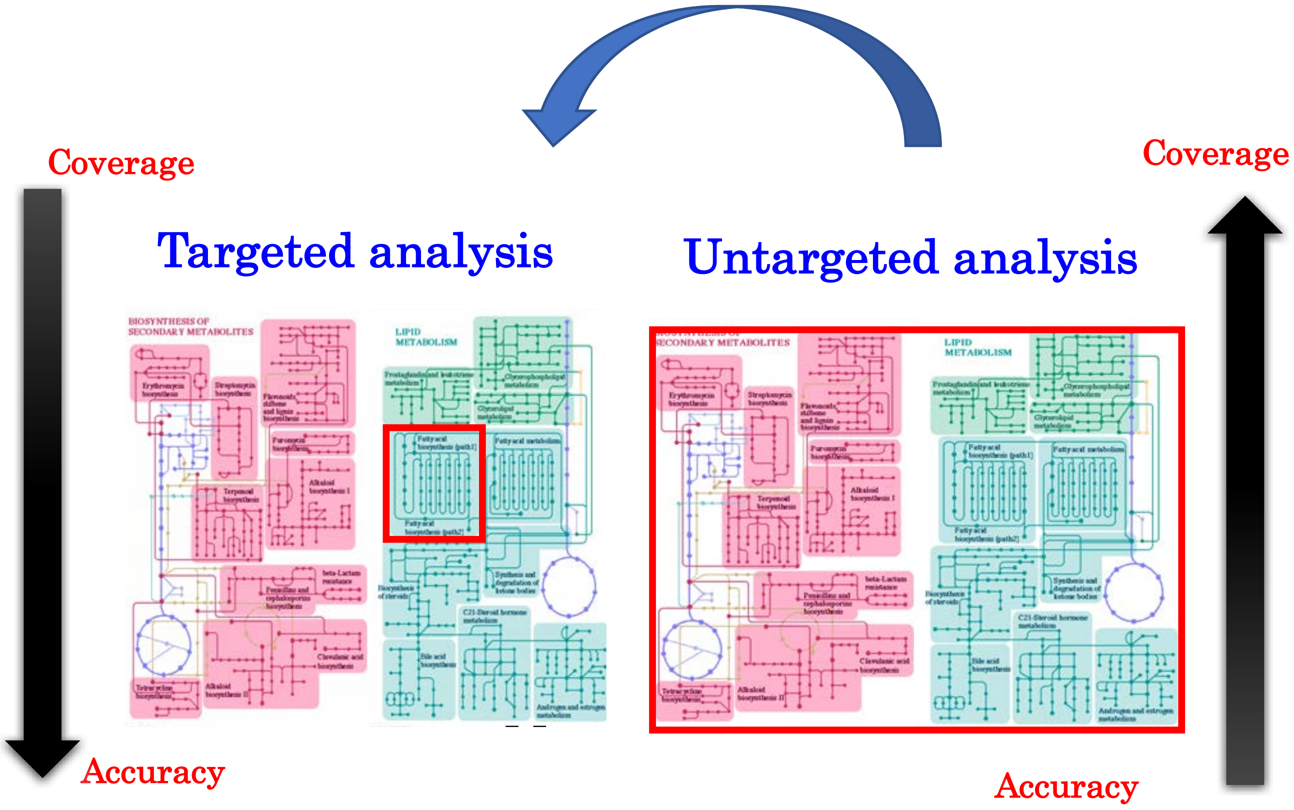
Dry chemistry





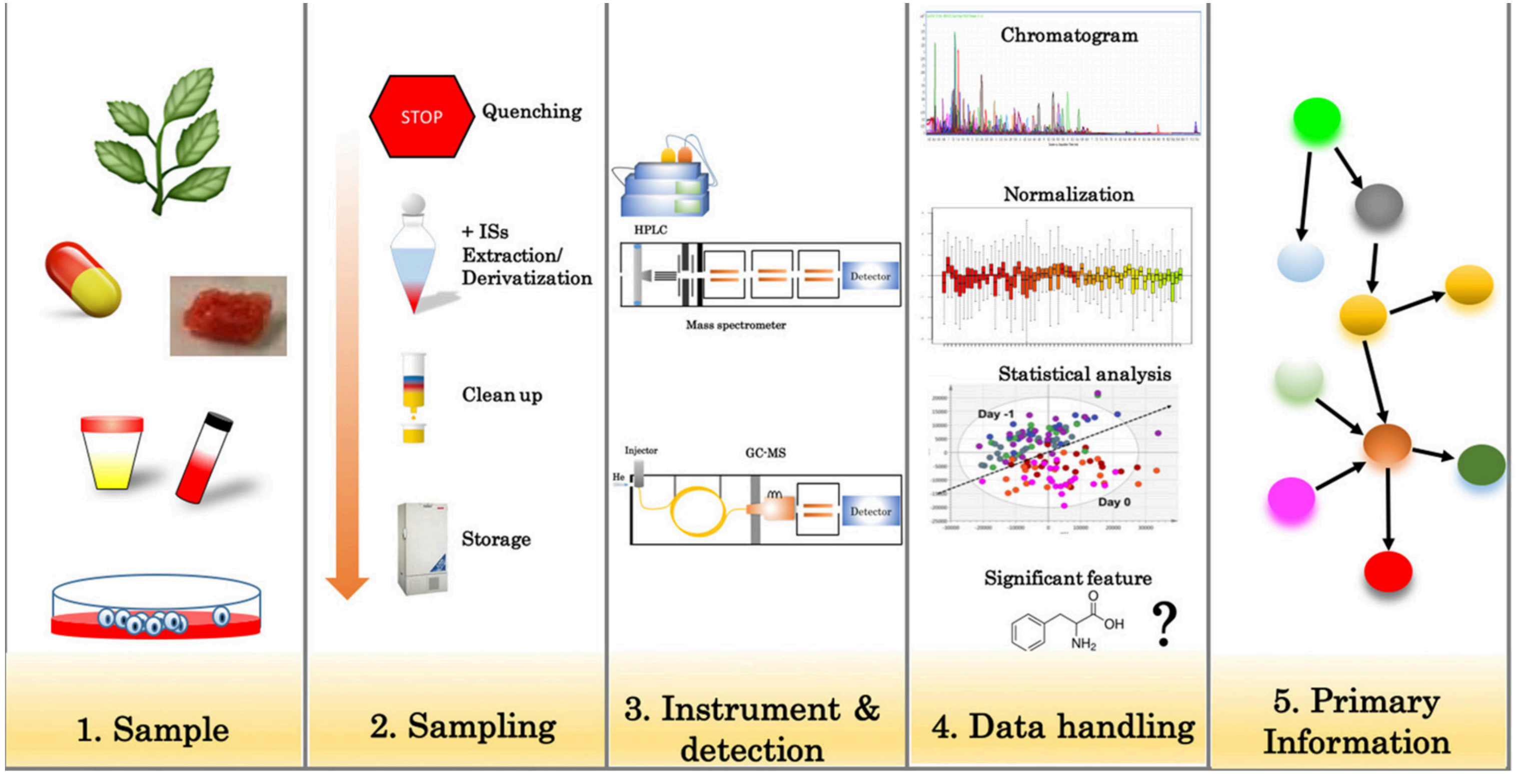
Approaches in metabolomics





Credit: Jens Nielsen, Chalmers University of Technology

Untargeted analysis (discovery)

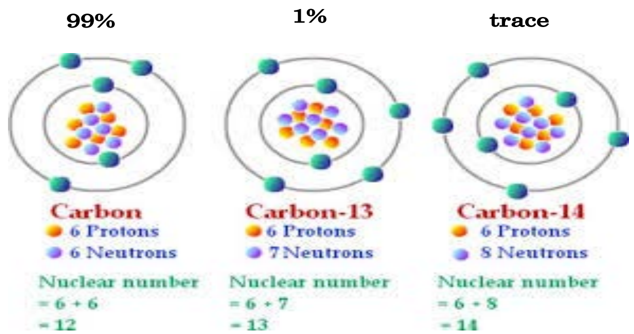


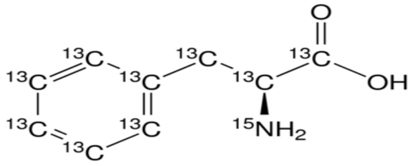
Deviation of retention time, sensitivity

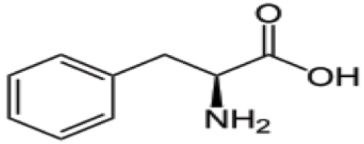
- Fluctuation of room temperature
- Pressure
- Column degradation
- Sample carryover
- Mobile phase

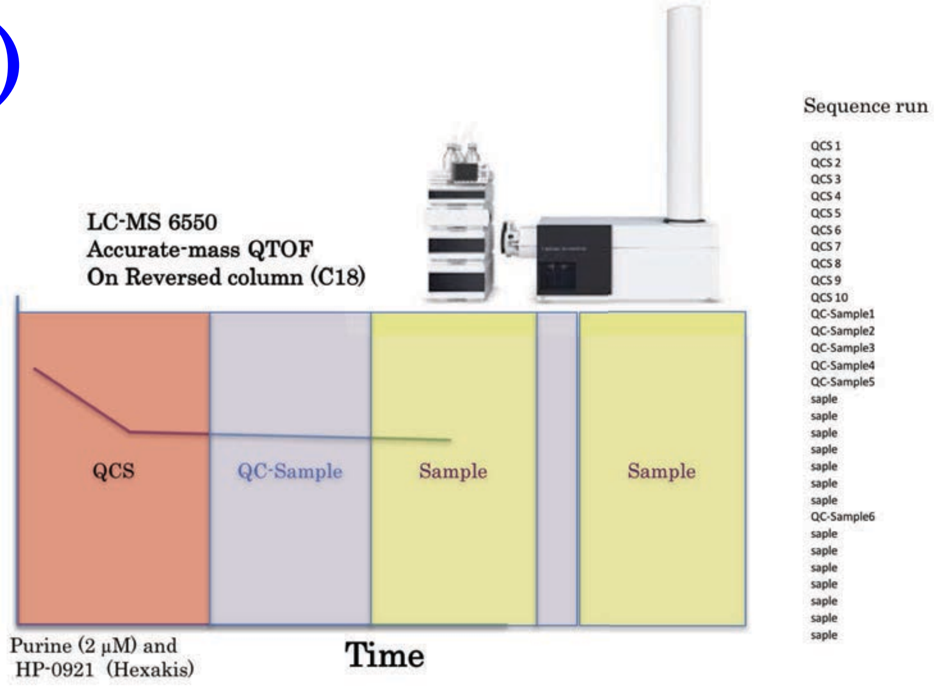
Untargeted analysis (continued)

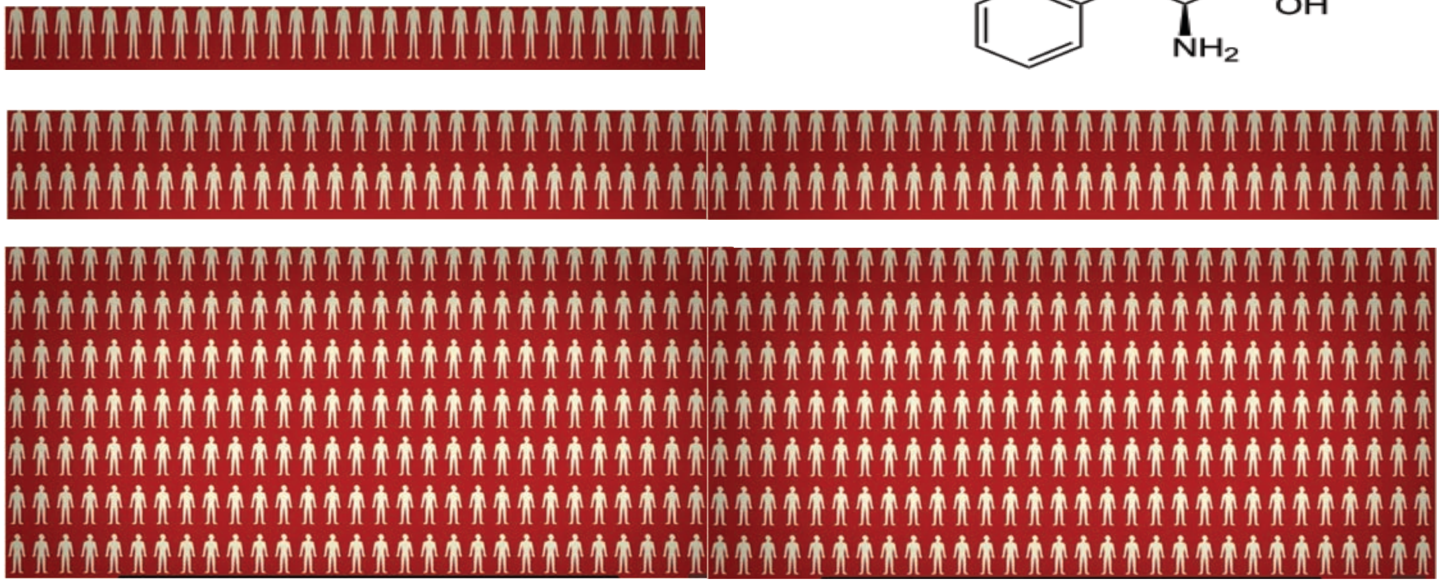
Sample extraction
Internal standard
Pooled sampled

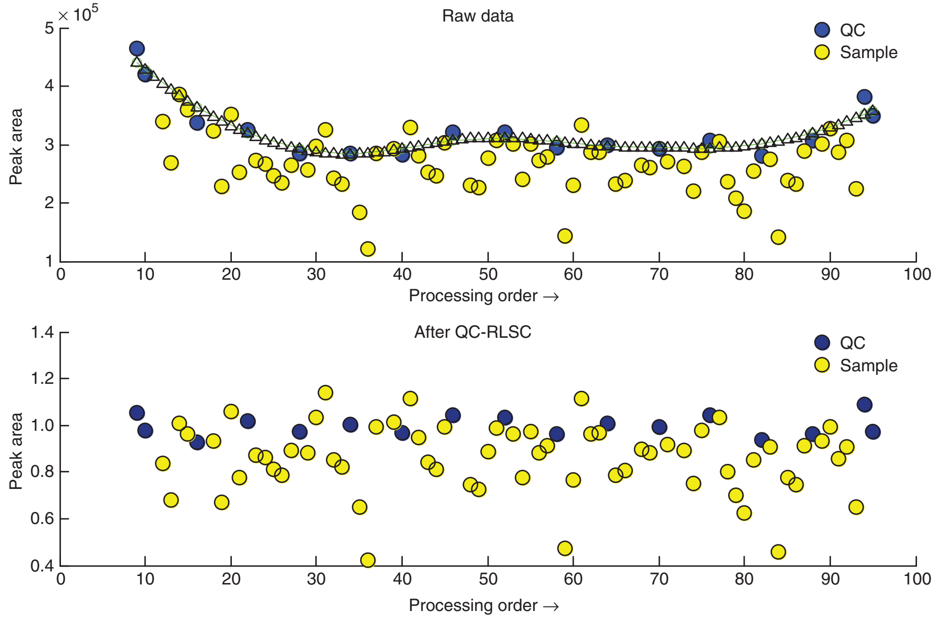






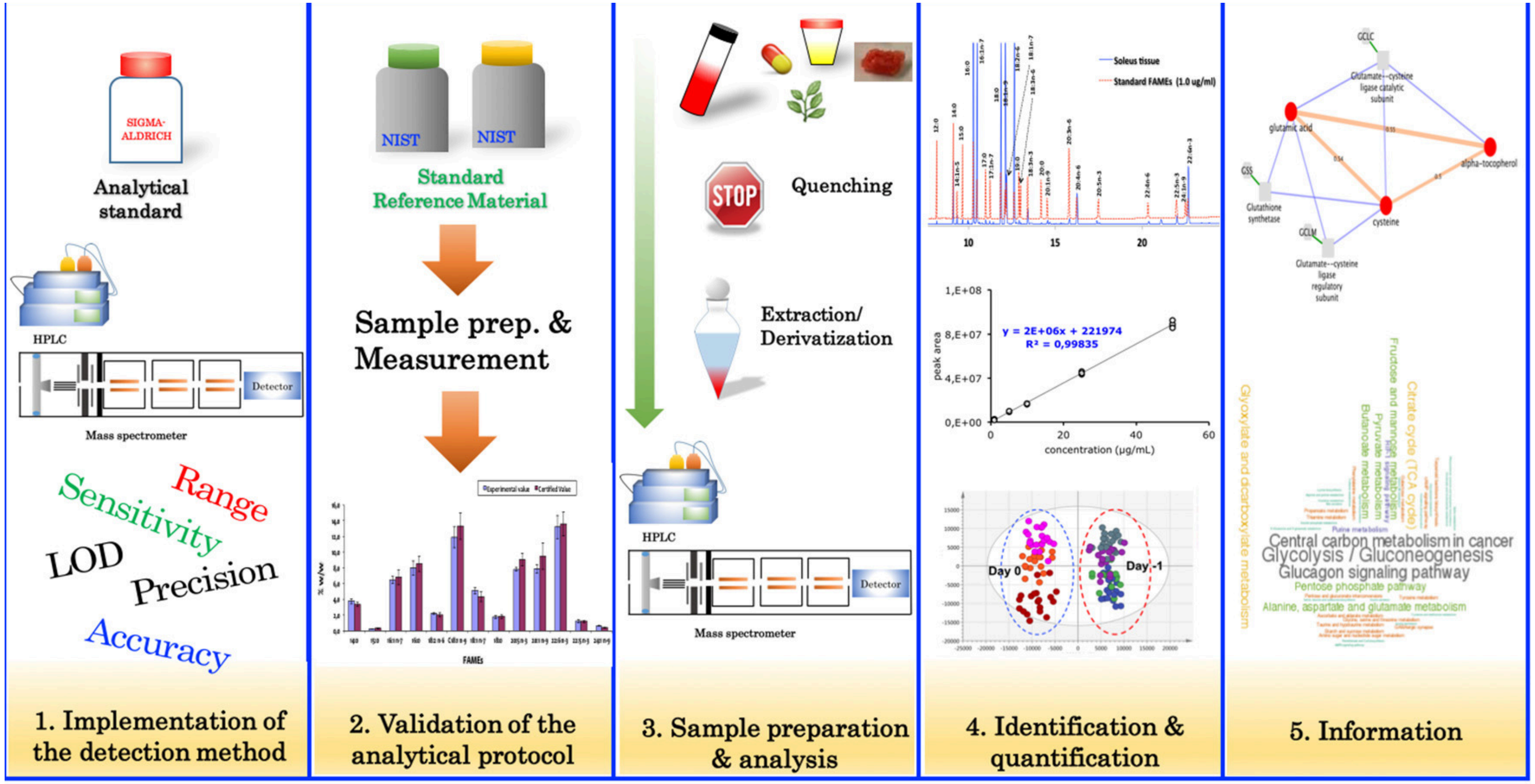






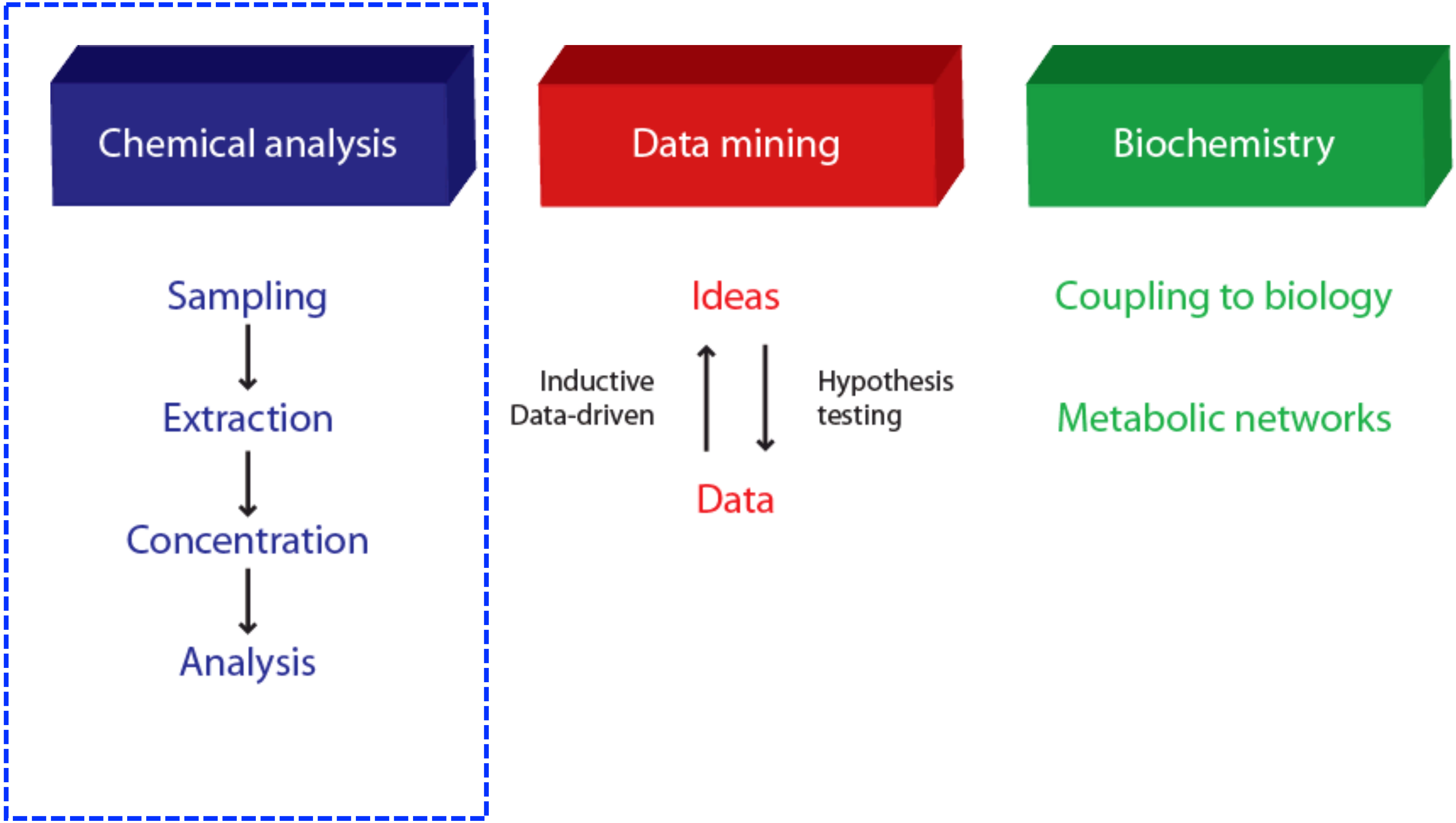
Targeted analysis (GC- or LC/MS)

- Confirm results from untargeted
- Quantitative or Qualitative
- Production for compounds of interest
- Pharmacokinetics studies
- Genetic modification on a specific enzyme

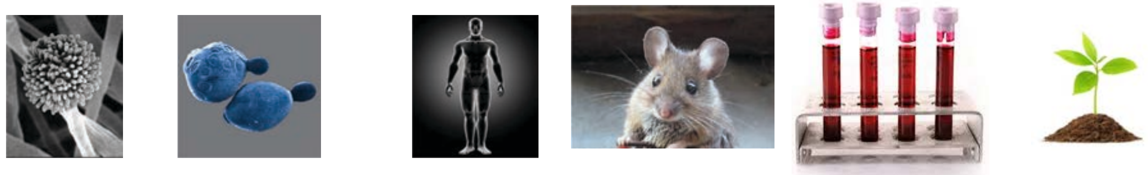


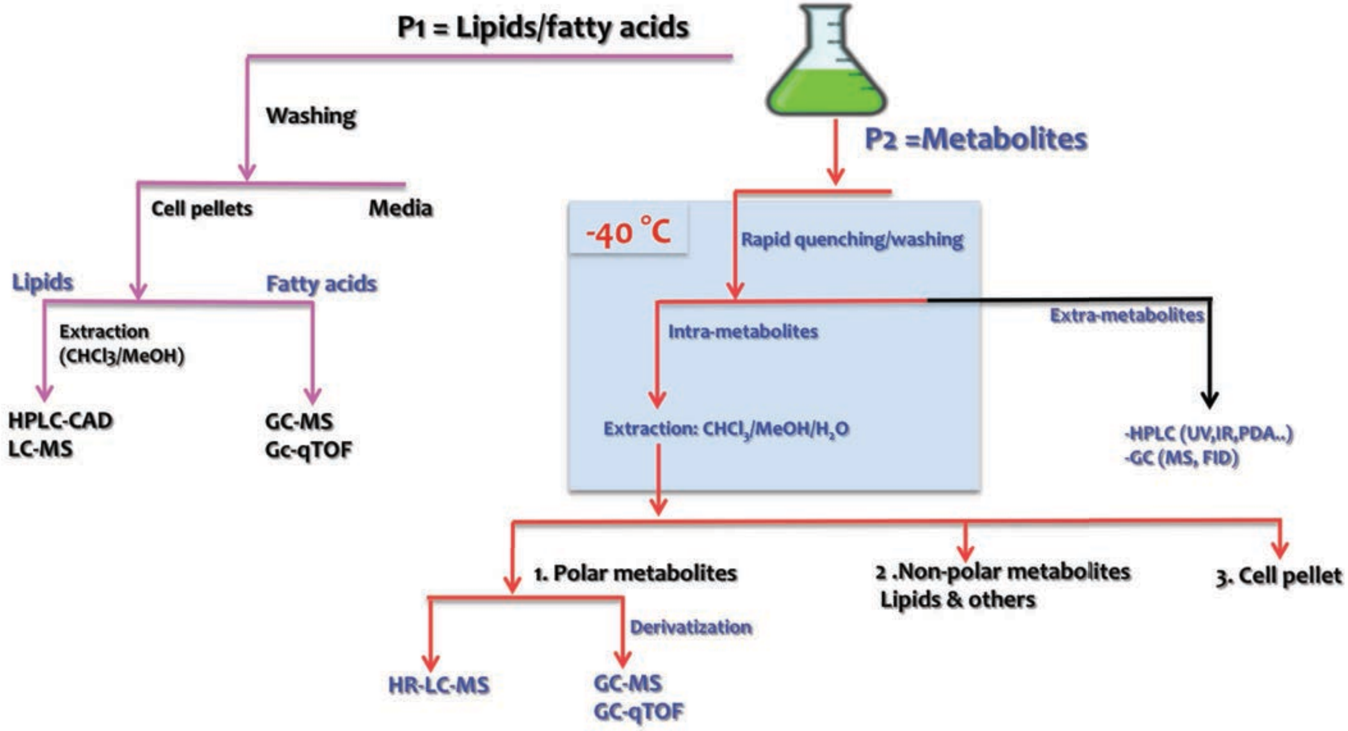
Front Pharmacol. (2017) 18;8:474.

Metabolomics workflow



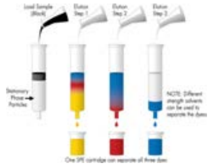
Chemical analysis (sampling & sample prep.)





Quenching

Extraction



Clean up/conc./
Derivatization

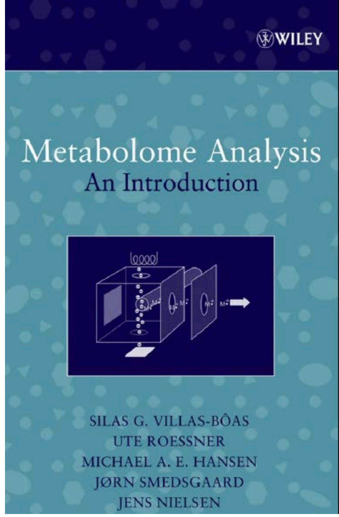
Storage

Analysis

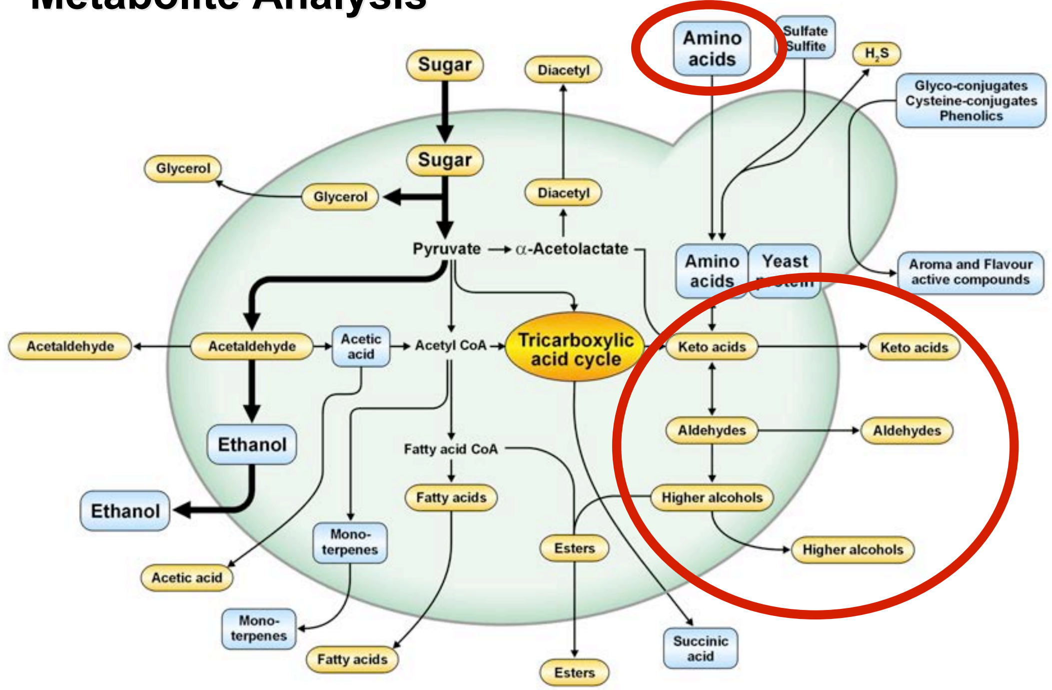
Why quenching

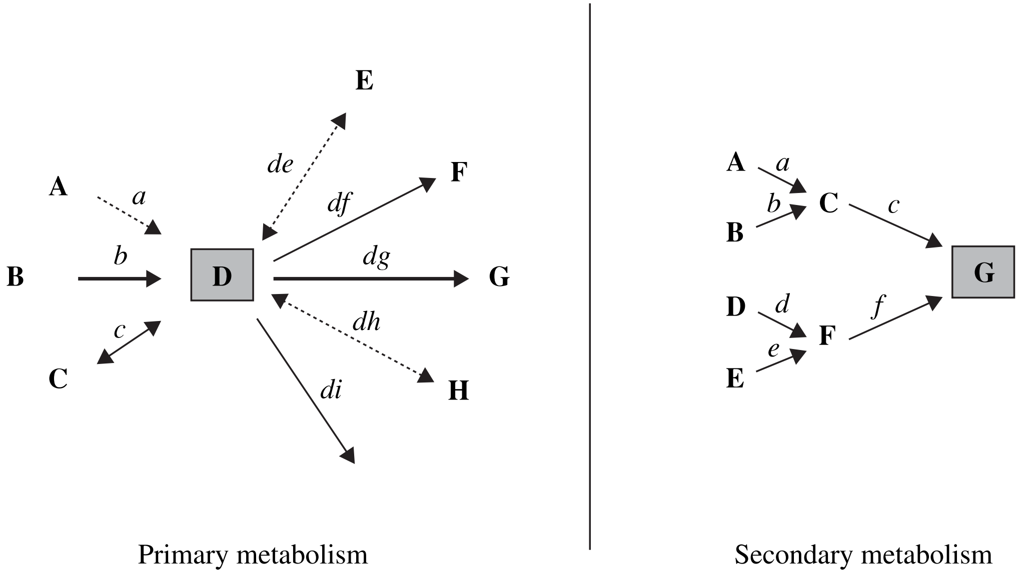
- Intracellular turnover
- Primary metabolite: metabolite that related to biochemical reaction
- Second metabolites: not used for the growth, secreted, or accumulated in the cell

- Extracellular turnover
- -Secreted metabolites



Metabolite Analysis



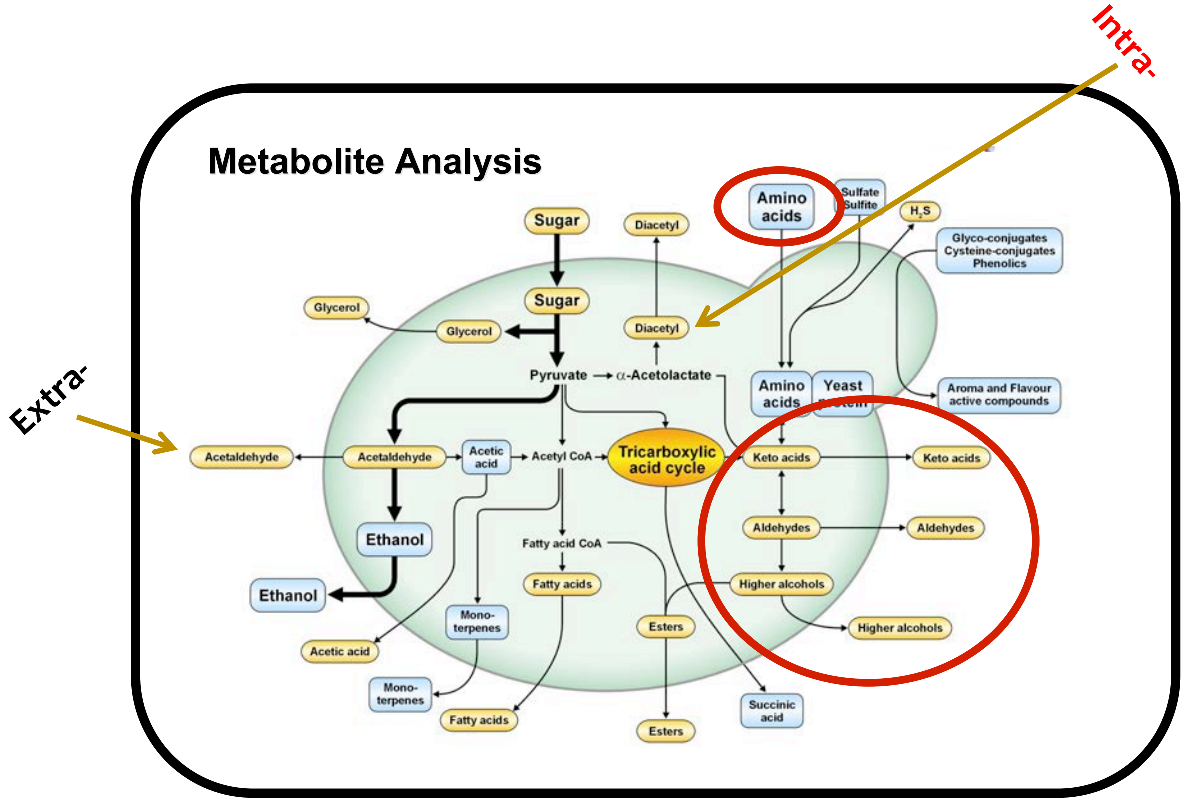


Quenching and washing (Metabolite)

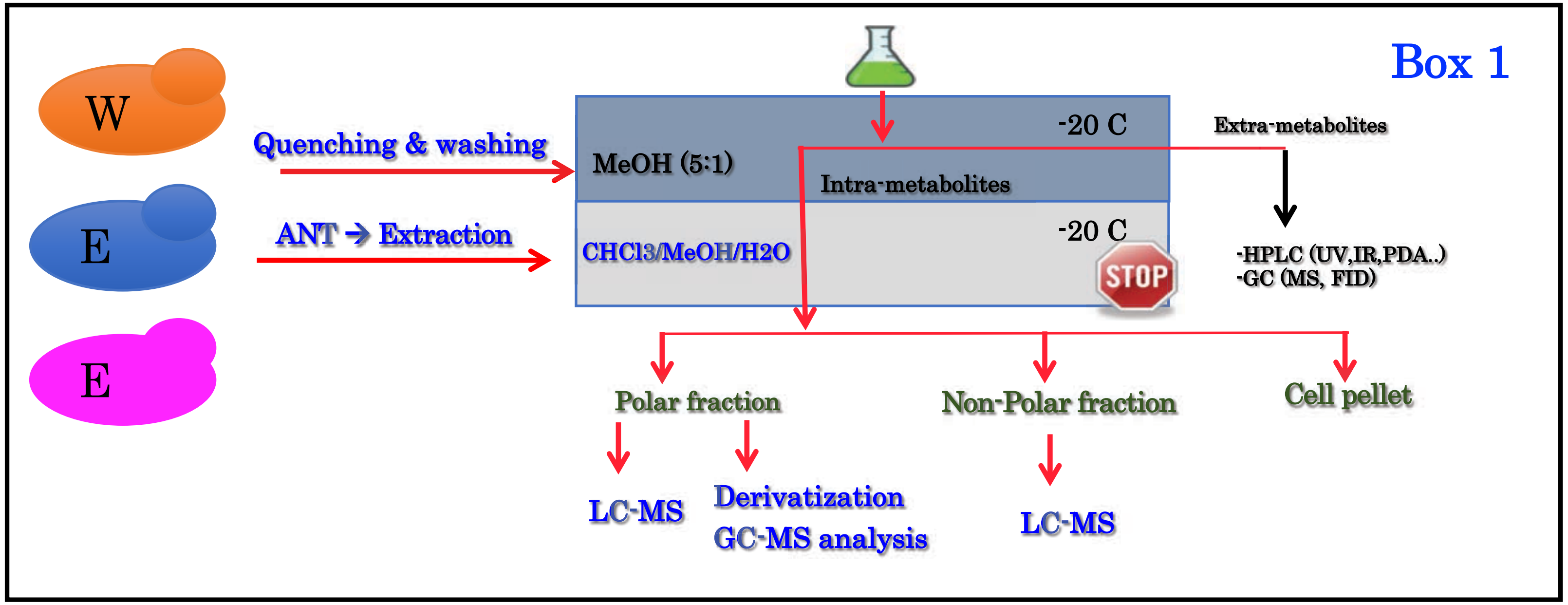
The purpose of quenching is to rapidly stop metabolism and Prevent change of metabolites

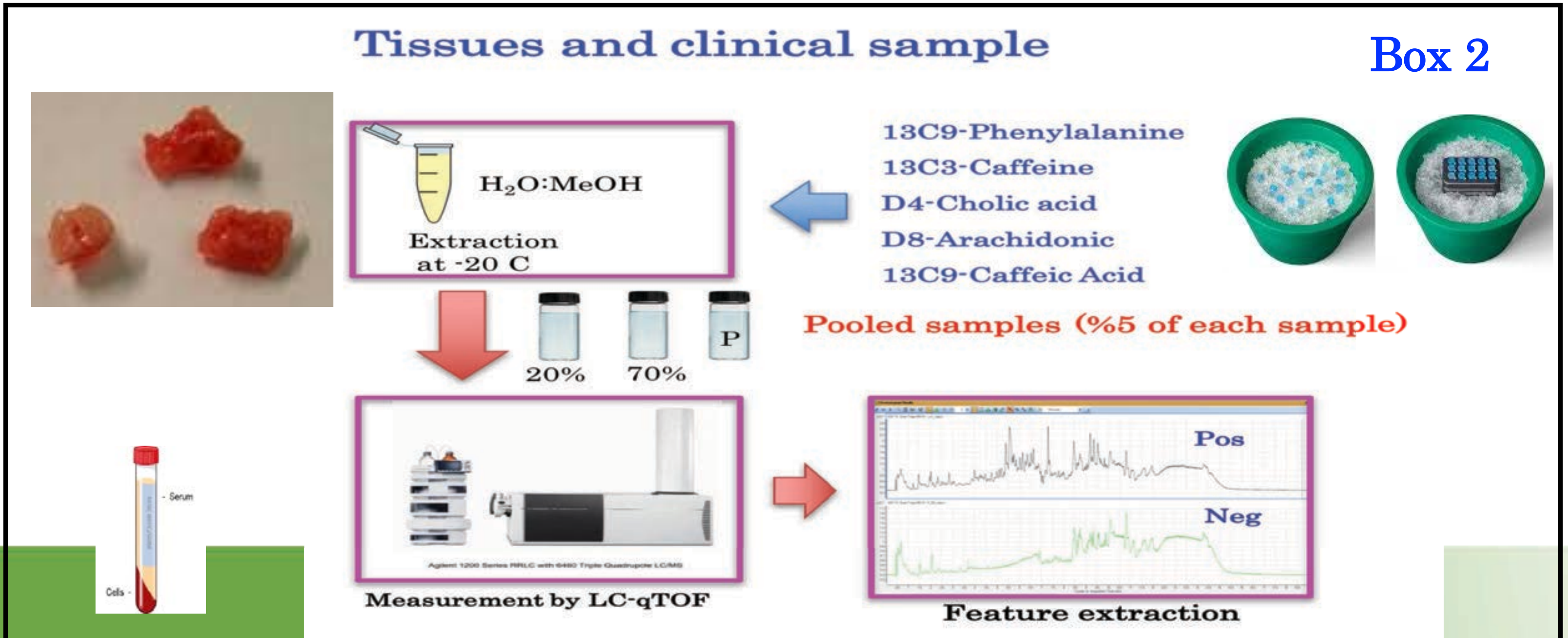
-Fast filtration and washing the cell with MEOH at -40 °C

-Cold method quenching at -40 °C



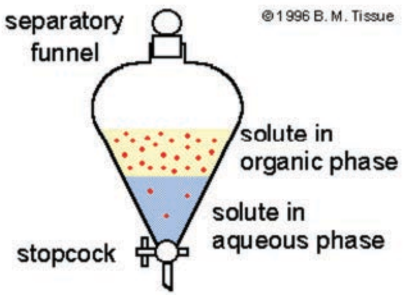
When is a good time for the internal standards

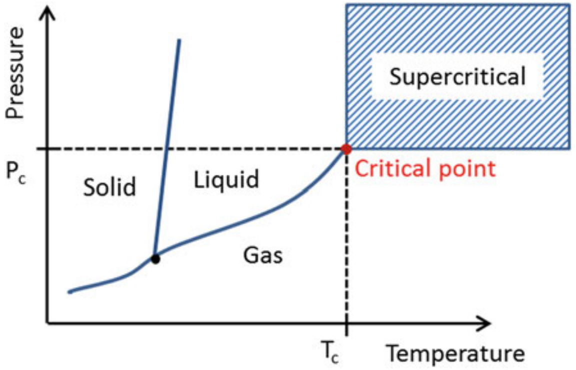


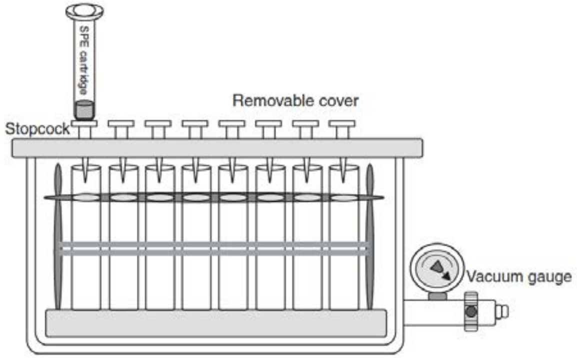


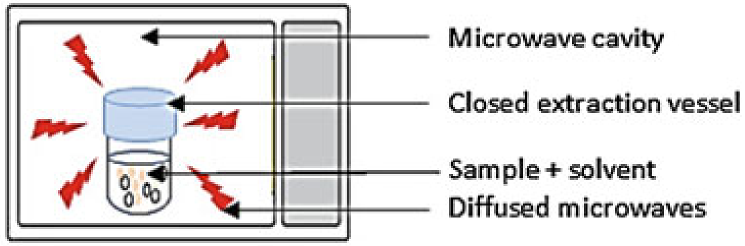
Extraction

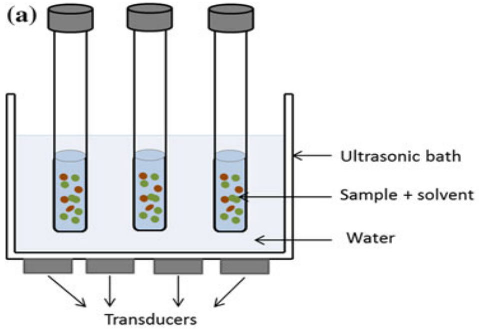
- Liquid-liquid extraction
- Solid-phase Extraction (SPE)
- Super critical fluid extraction
- Ultrasound Extraction
- Microwave-assisted extraction











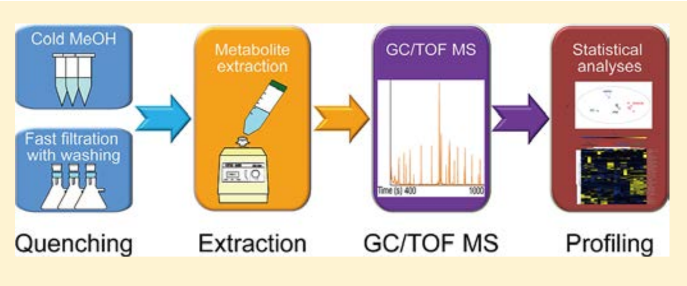
analytical chemistry

Article

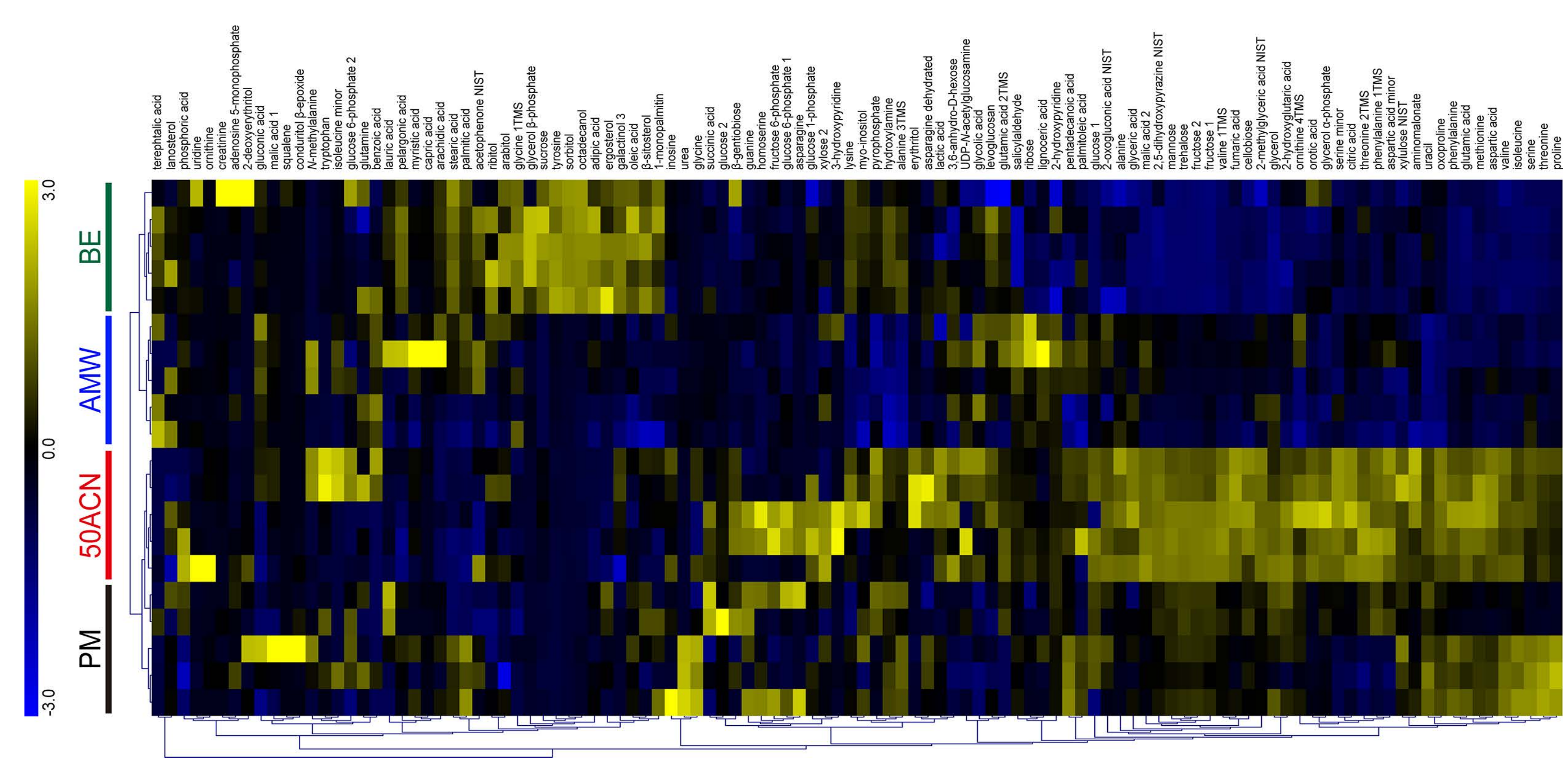
pubs.acs.org/ac

Evaluation and Optimization of Metabolome Sample Preparation Methods for *Saccharomyces cerevisiae*

Sooah Kim,† Do Yup Lee,‡ Gert Wohlgemuth,§ Hyong Seok Park,† Oliver Fiehn,§ and Kyoung Heon Kim*,†

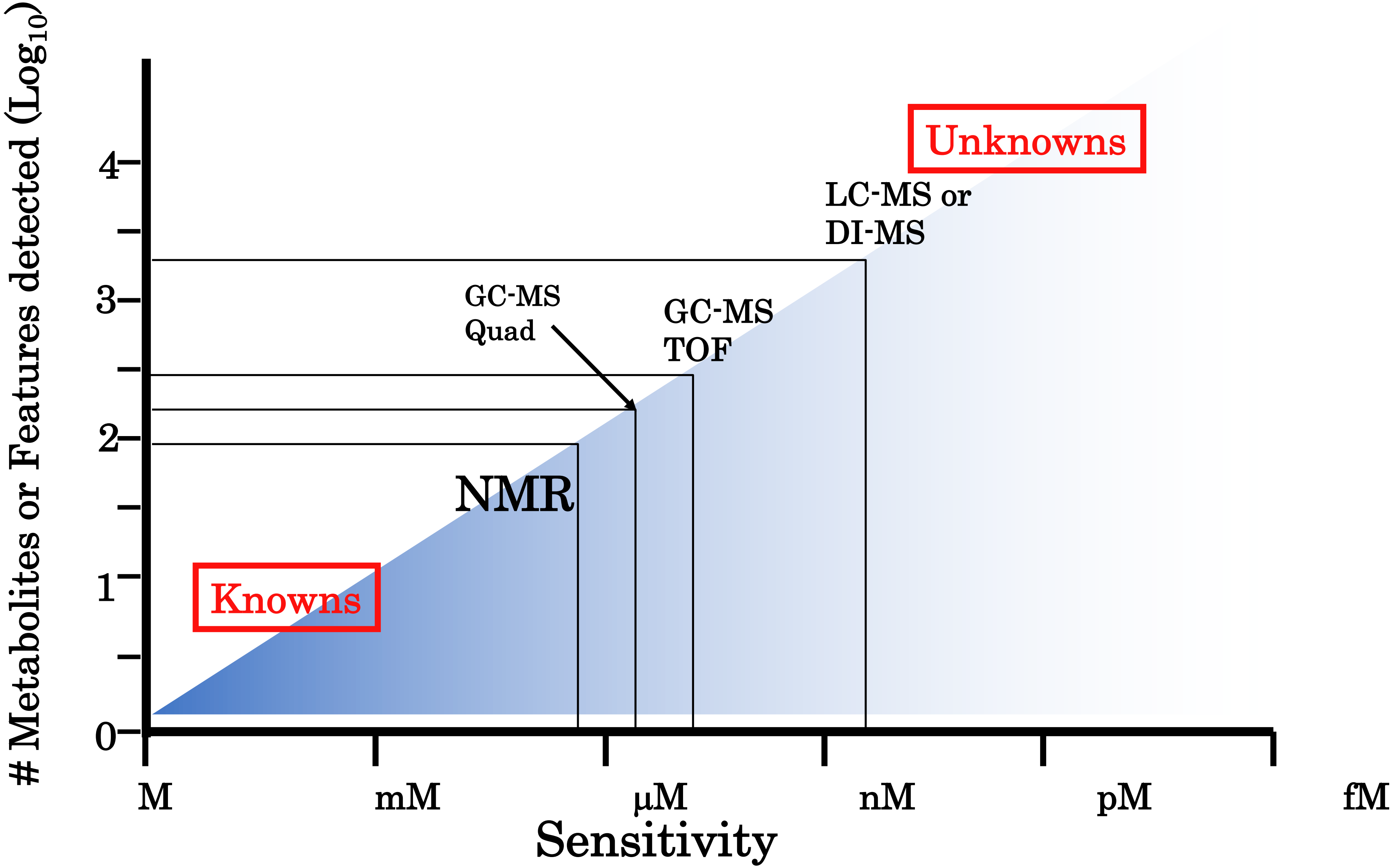


PM = Pure Methanol
50ACN = 50% Acetonitrile
AMW = Acetonitrile/methanol/ water
Boiling Ethanol



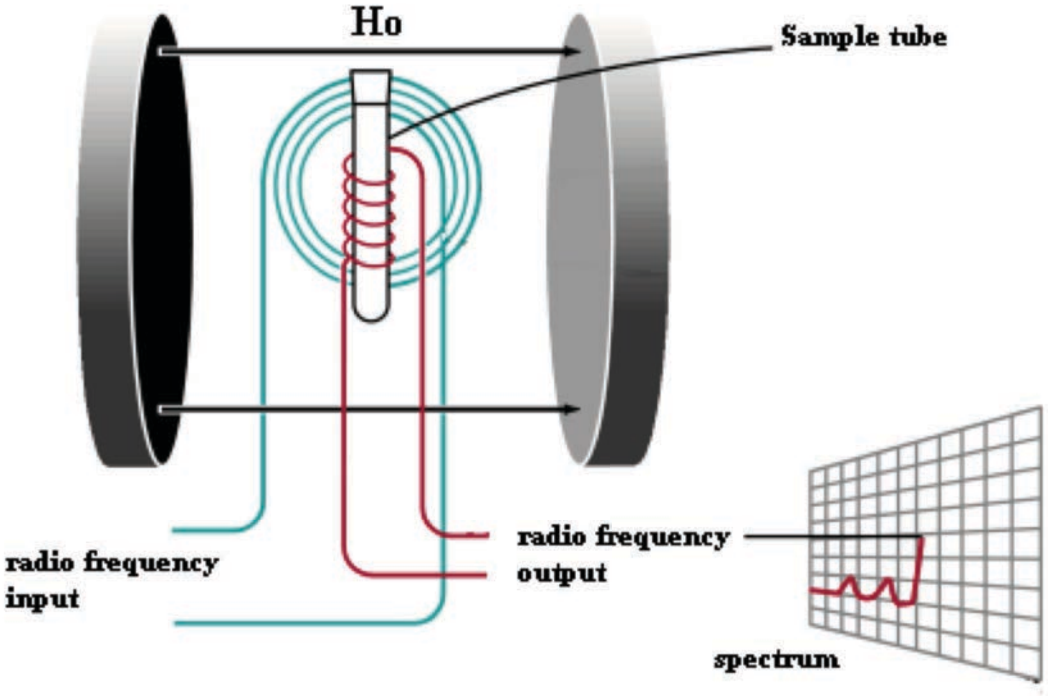
Siriraj Metabolomics & Phenomics Center

Technology & Sensitivity



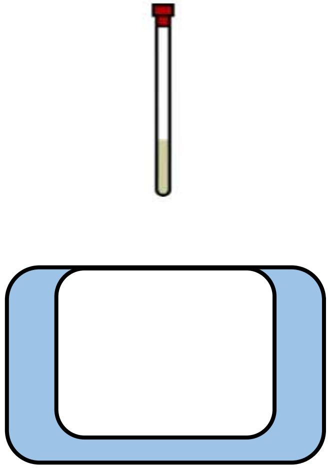
Credit: David Wishart, University of Alberta, Canada

Nuclear magmatic resonance (NMR)



https://en.wikipedia.org/wiki/Nuclear_magnetic_resonance

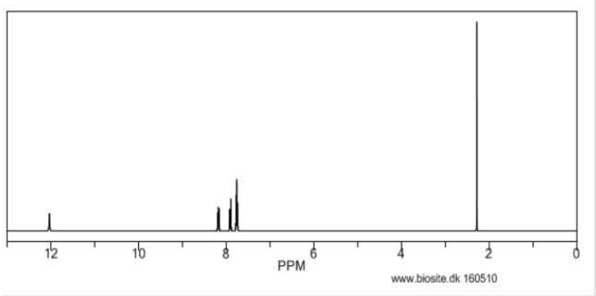
https://orgspectroscopyint.blogspot.com/2015/06/nuclear-magnetic-resonance-spectroscopy.html



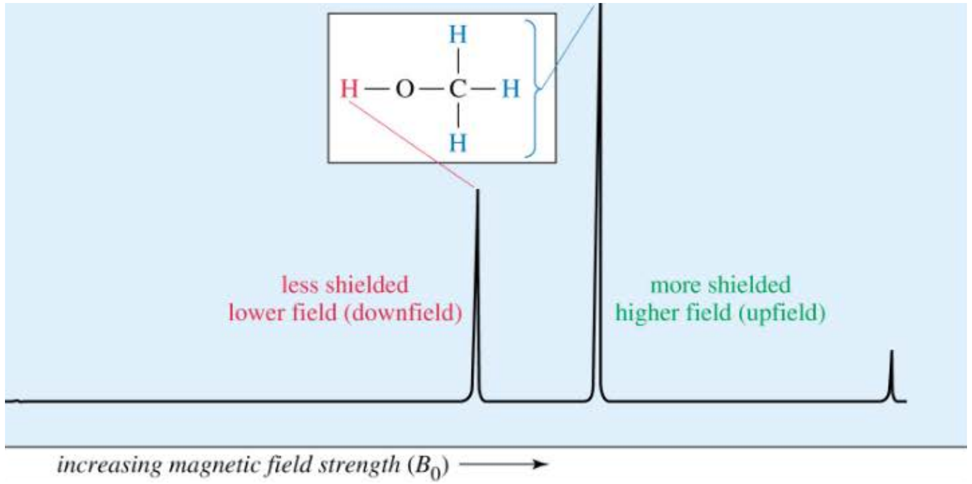
Strong magnetic field

Apply energy

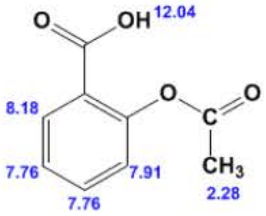
(Radio frequency)



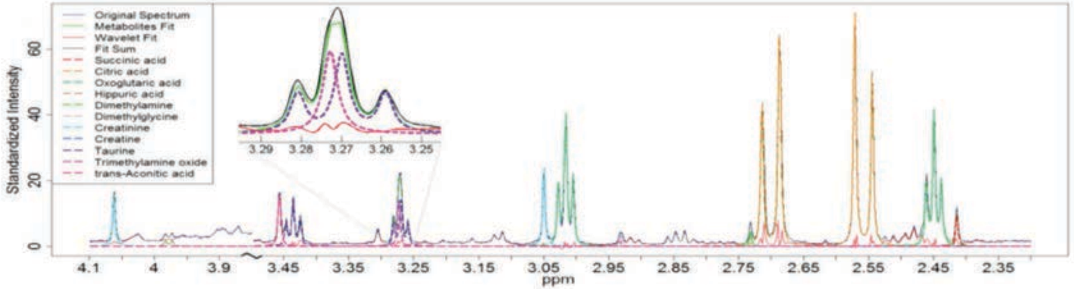
NMR spectrum



Interpret

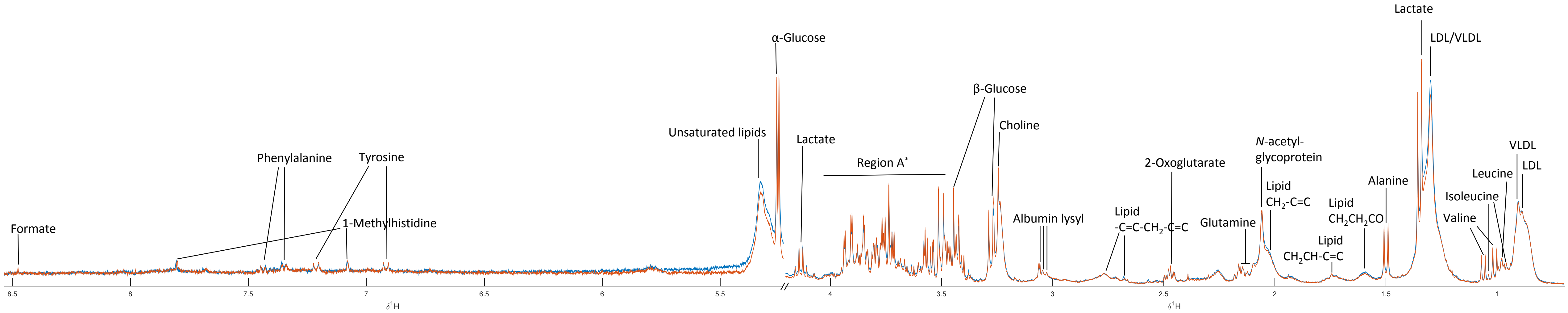


Structure



Dr. Sakchai Hongtong

Serum and Plasma CPMG NMR Spectra

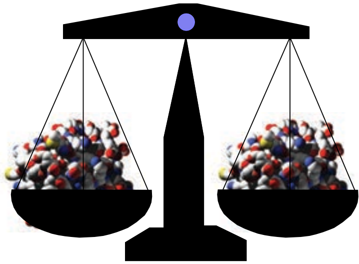


Analyst: Jutarop Phetcharaburanin (21/05/2018)
Spectrometer: Bruker Ascend 400 MHz (128 scans)
Sample: Fasting serum (blue) and plasma (red)
Spectra: CPMG spin-echo
Metabolite assignment:

- Imperial College London's in-house database
- Chenomx Profiler
- STOCSY
- Nicholson *et al.* (1995), Anal Chem.
- Phetcharaburanin *et al*. (2016), J Proteome Res.

*Region A contains signals from glycerol, glucose and amino acid CH protons.
To identify these signals, 2-D NMR and spike-in of standard chemical experiments are required.

Mass spectrometry (MS)



John B. Fenn

Nobel prize winner 2002

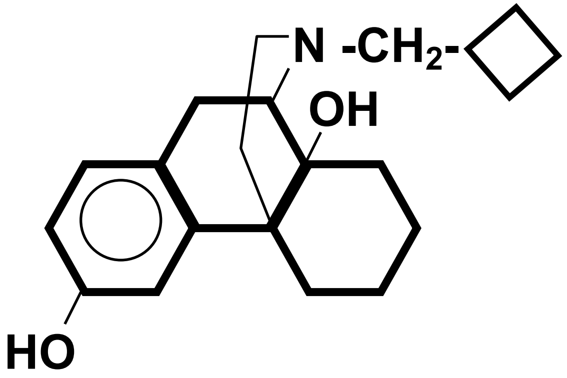
"Mass spectrometry is the art of measuring atoms and molecules to determine their molecular weight. Such mass or weight information sometime sufficient, frequently necessary and always useful in determining the identity of a species..."

1. Hyphenated techniques
   - -GC-MS
   - -LC-MS
   - -CE-MS
2. Direction injection mass spectrometry

MS Principles

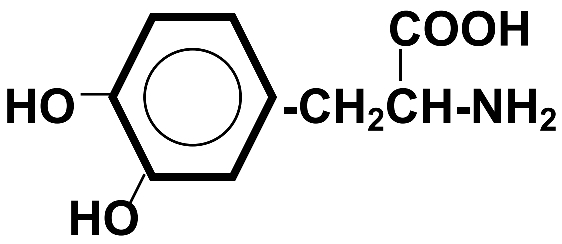
- Different compounds can be uniquely identified by their mass

Butorphanol



MW = 327.1

L-dopa



MW = 197.2

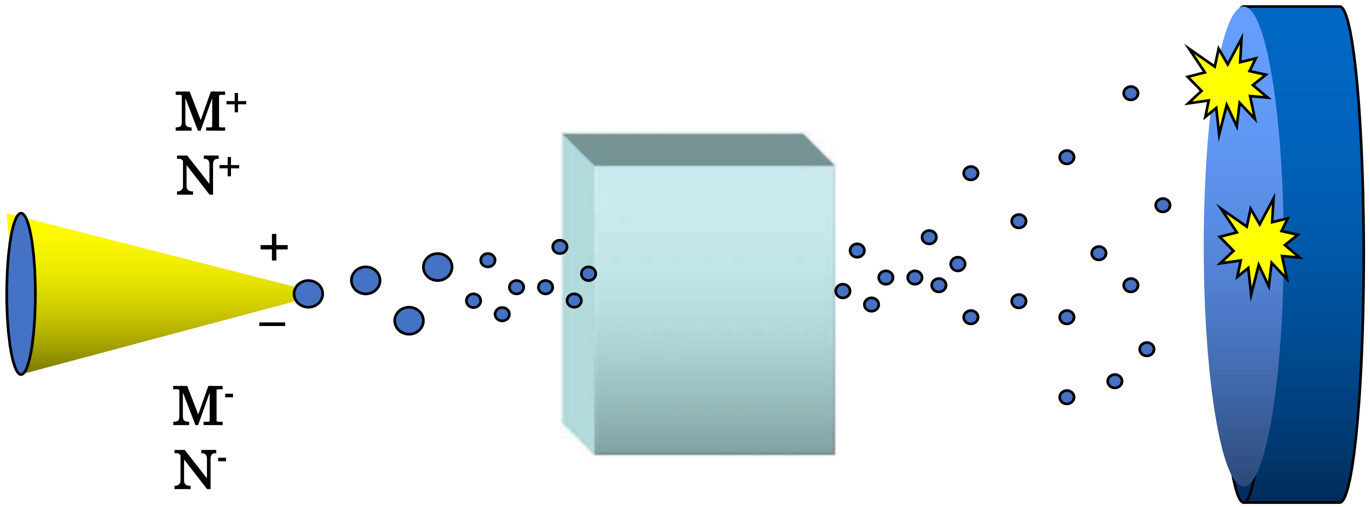
Ethanol

CH_3CH_2OH

MW = 46.1

Mass Spec Principles

M
N



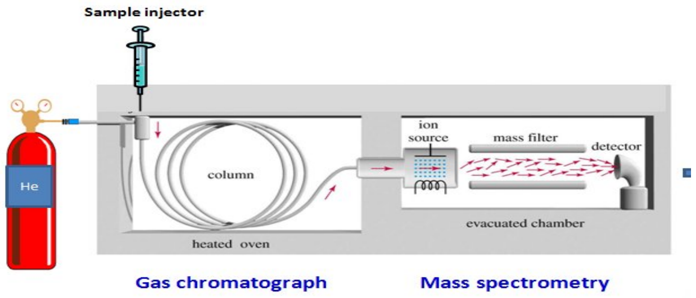
Sample inlet
GC
LC

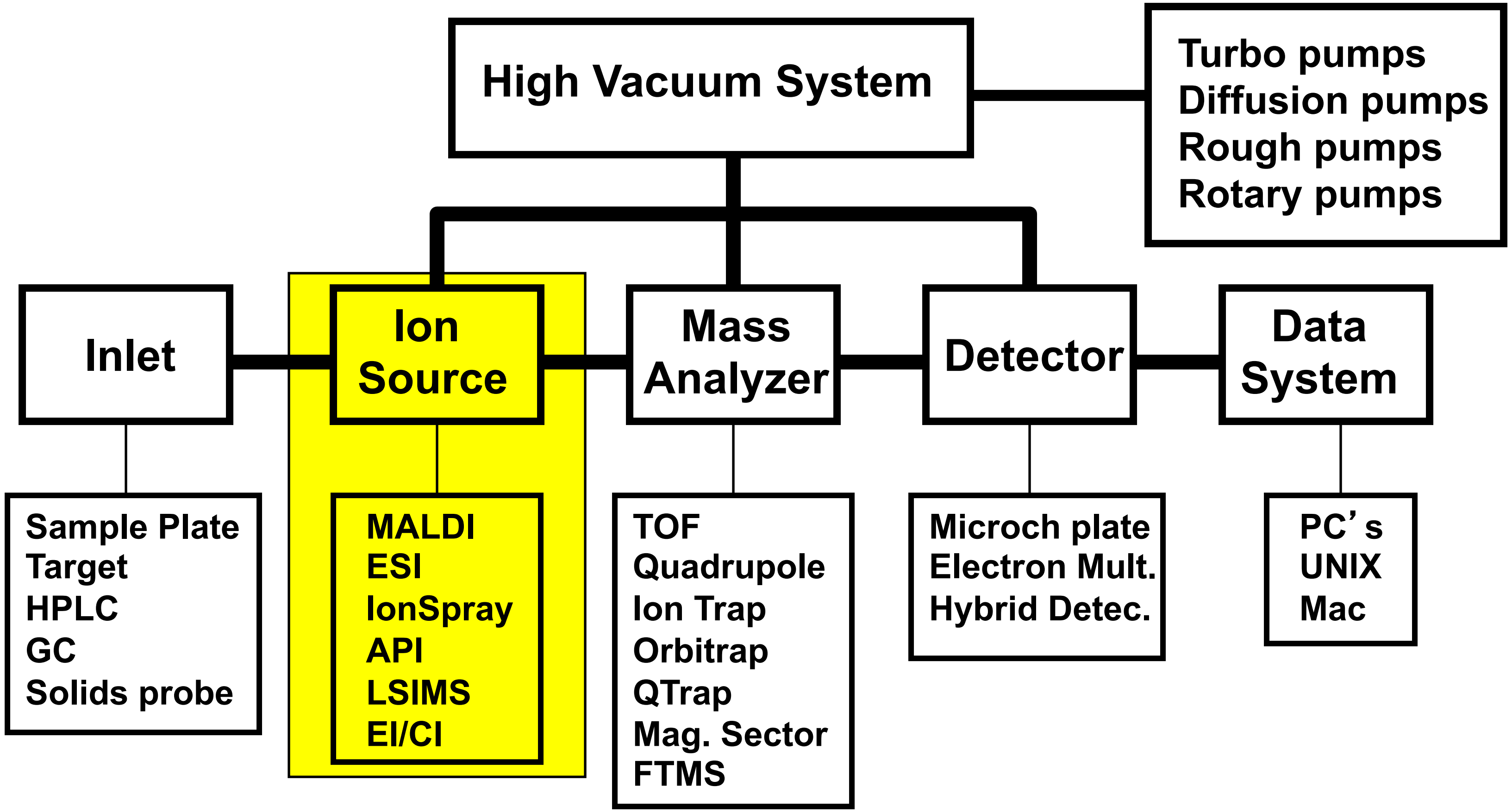
Ionizer

Mass Analyzer

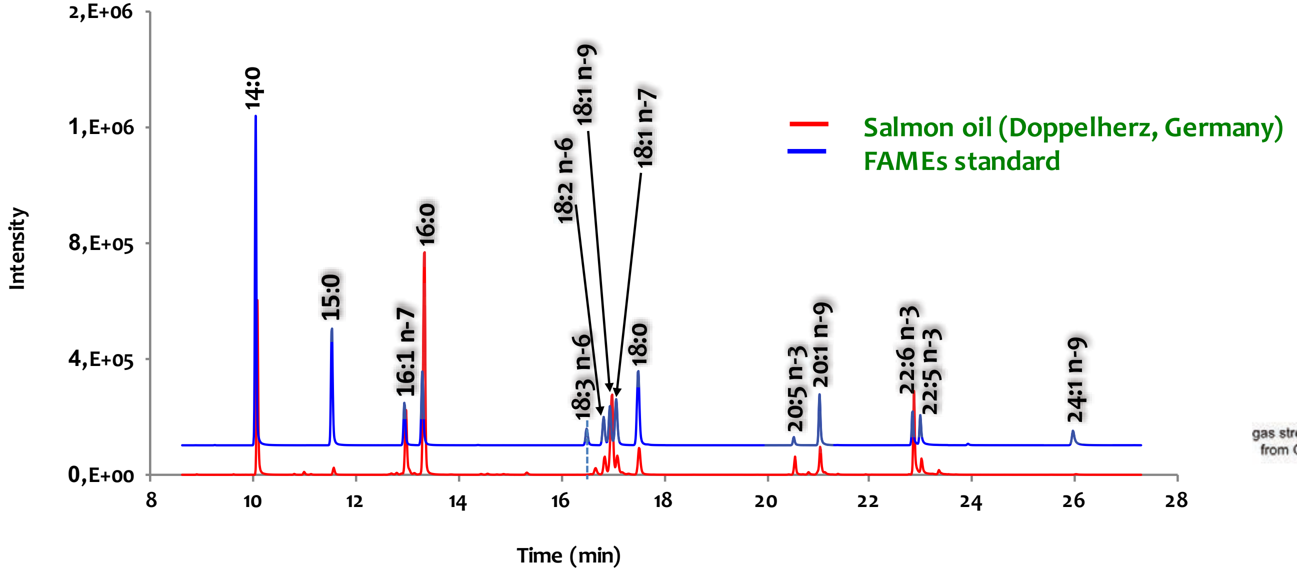
Detector

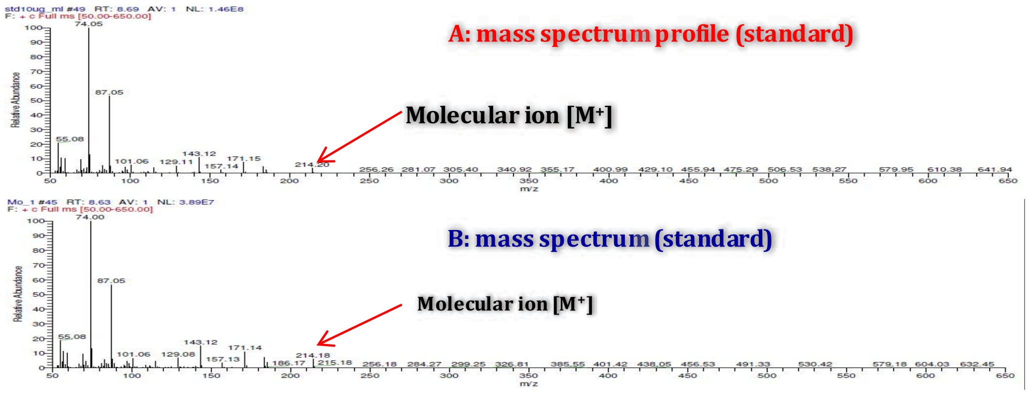
Mass Spectrometer Schematic



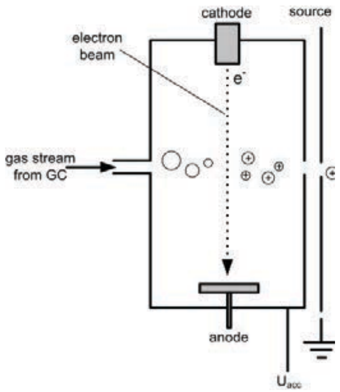


Output

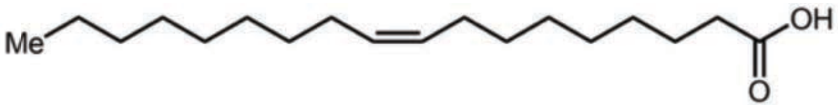




GC-EI-MS

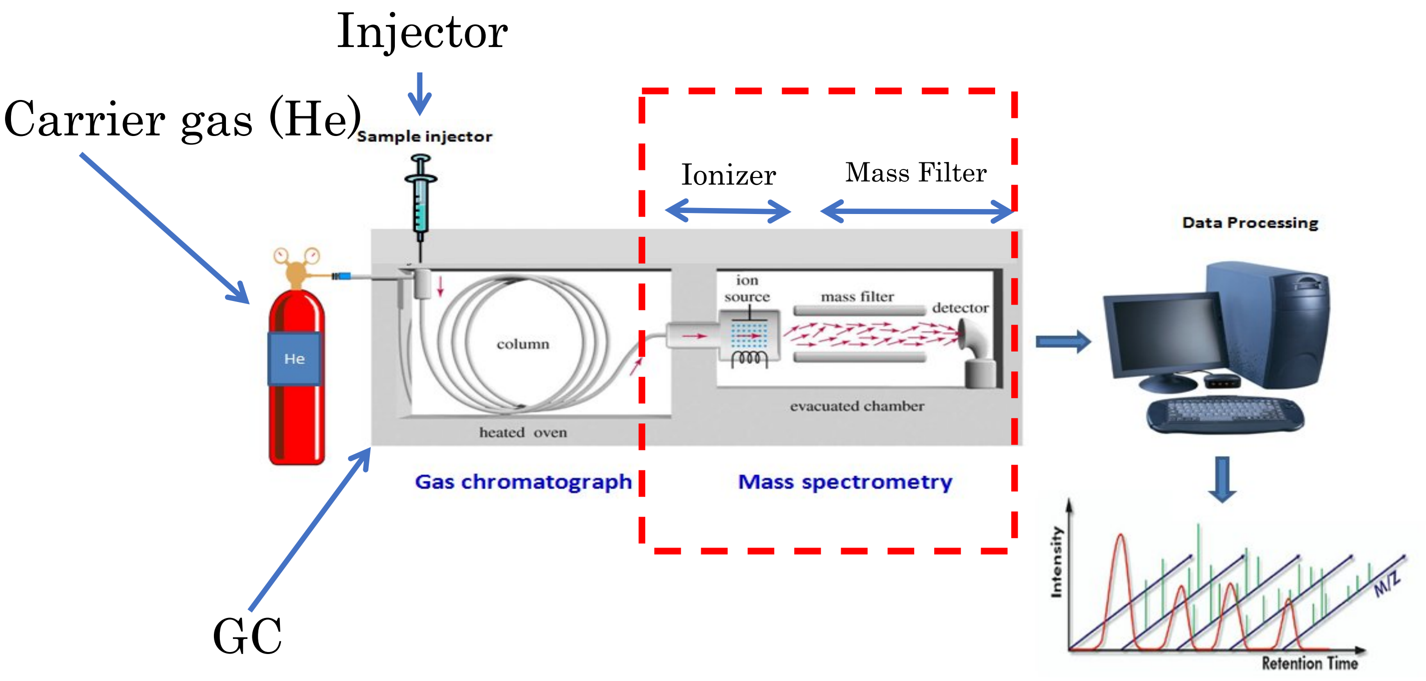


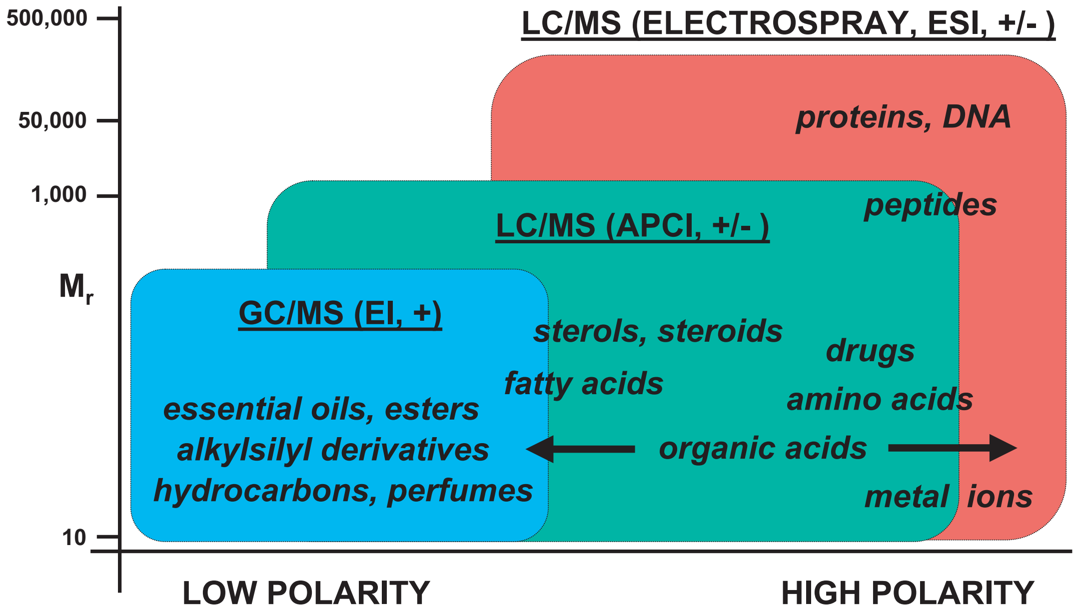
EI = Electron ionization



Oleic acid (18:1 n=9)

GC-MS

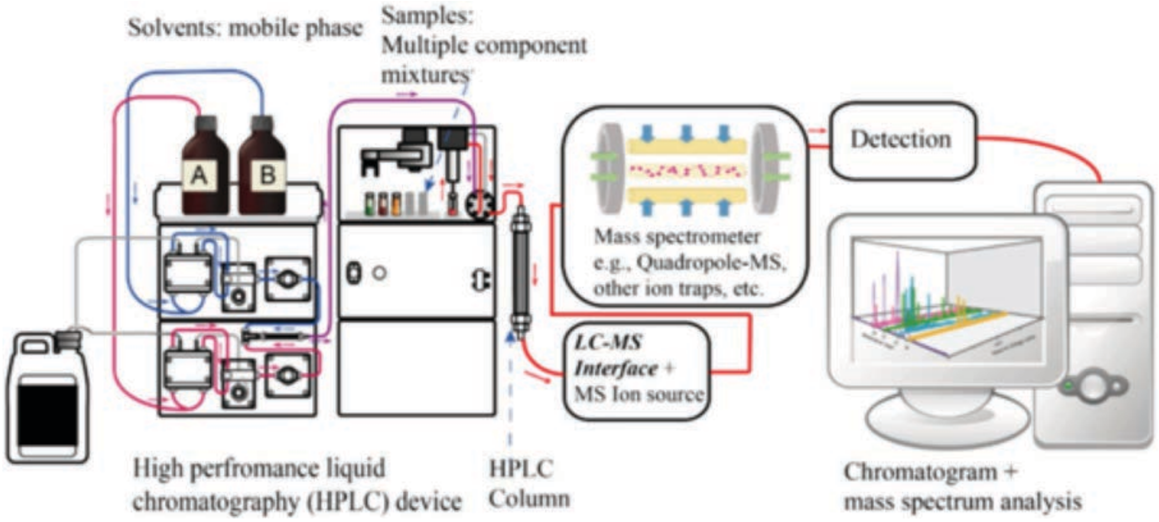


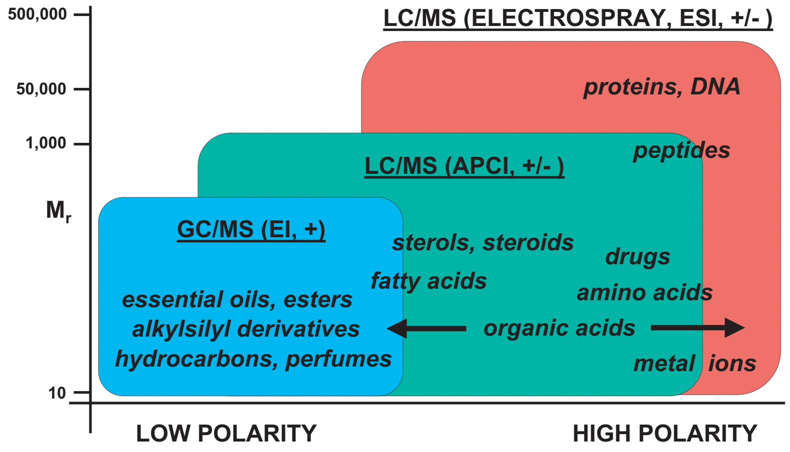


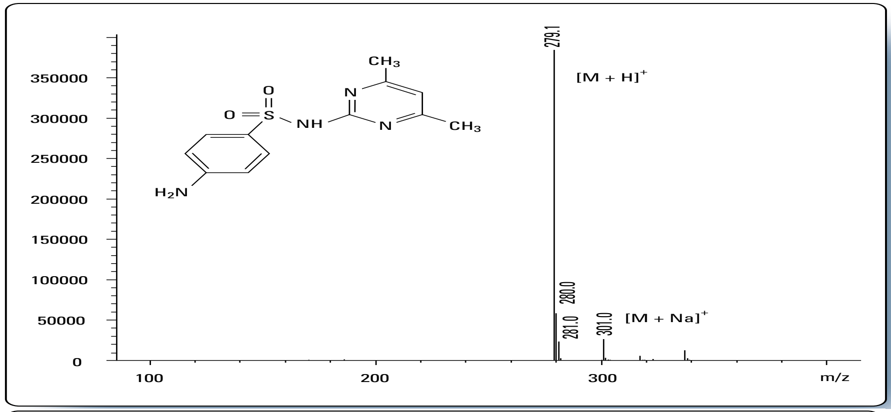
1. Mobile phase: He and H_2
2. GC: Column: polar, mid-polar and non-polar
3. Ionizer: Electron ionization (EI), Chemical Ionization (CI)
4. Mass analyzer: Quadrupole (S and T), TOF or Q-TOF

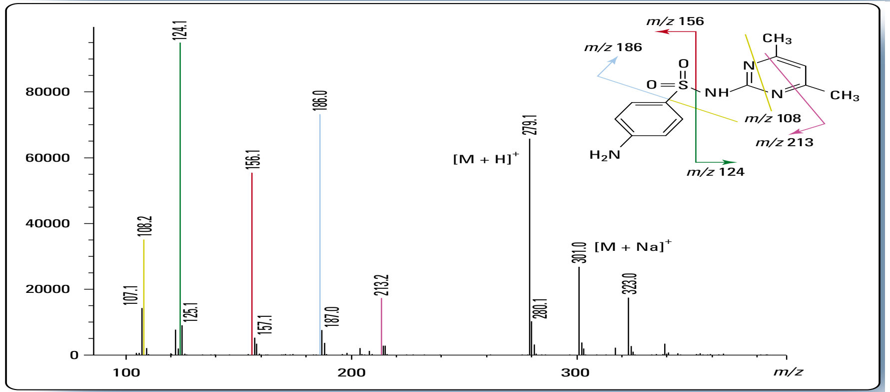
J. Exp. Bot., 2005, 56, 219–243.

Liquid Chromatography MS (LC-MS)







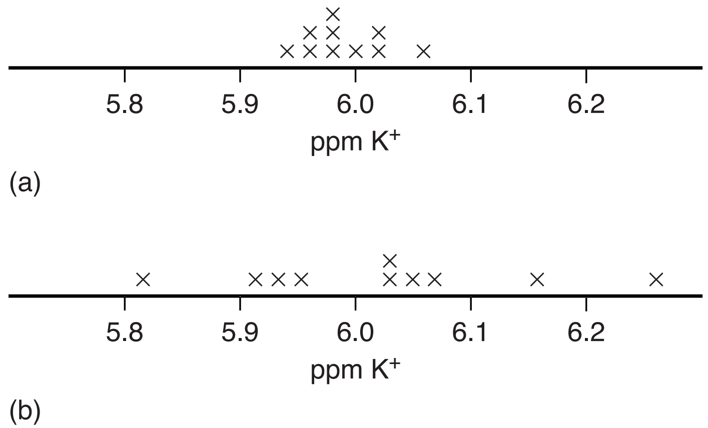


Accuracy and precision

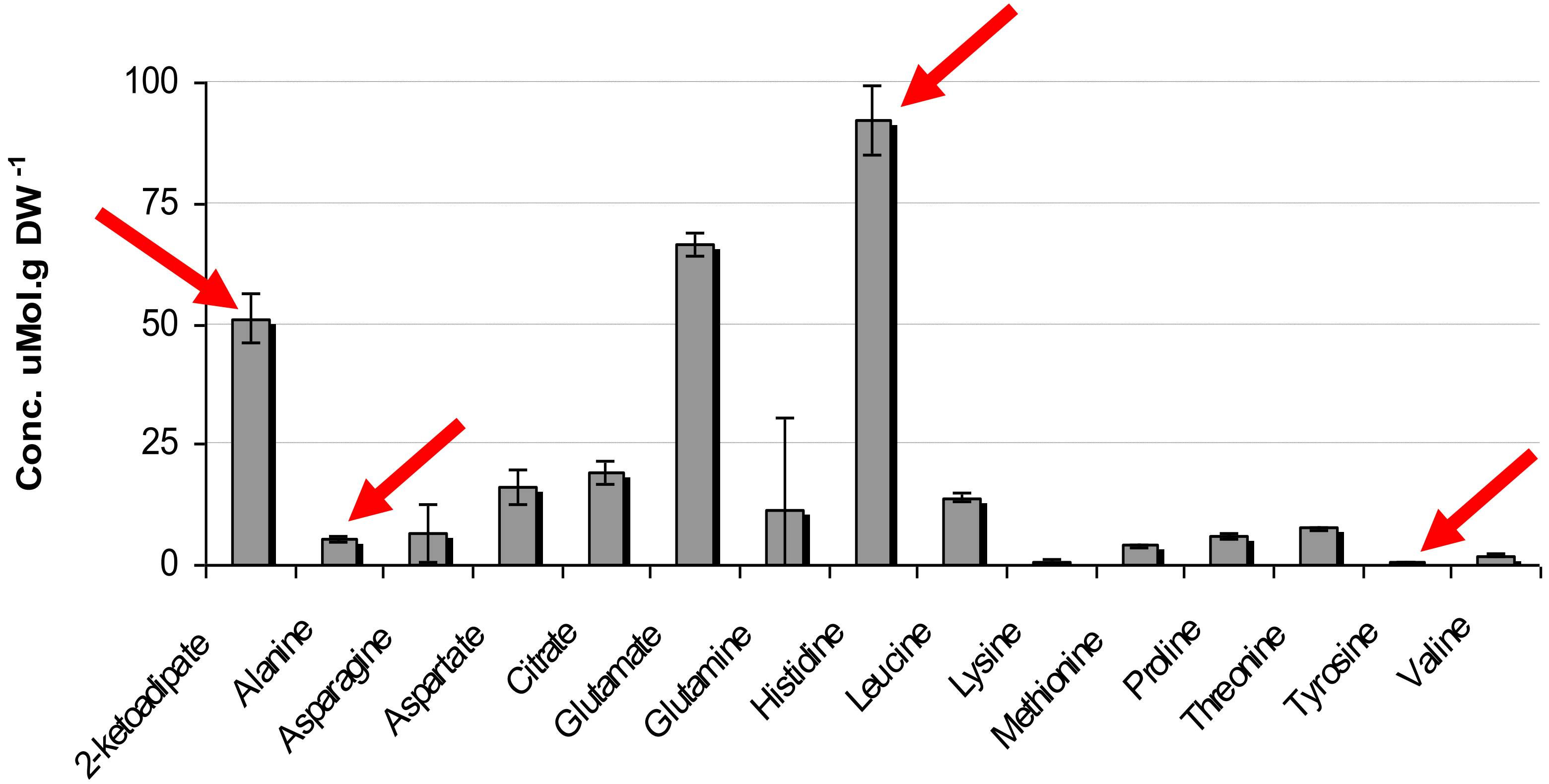
Accuracy: % Error = (Obtained result – expected result)/expected X 100

- Standard reference material (National Institute of Standard and Technology
  - Spiking experiment

Precision: A measure of variability = SD/mean X 100 (%RSD)



Good Reproducibility (precision)



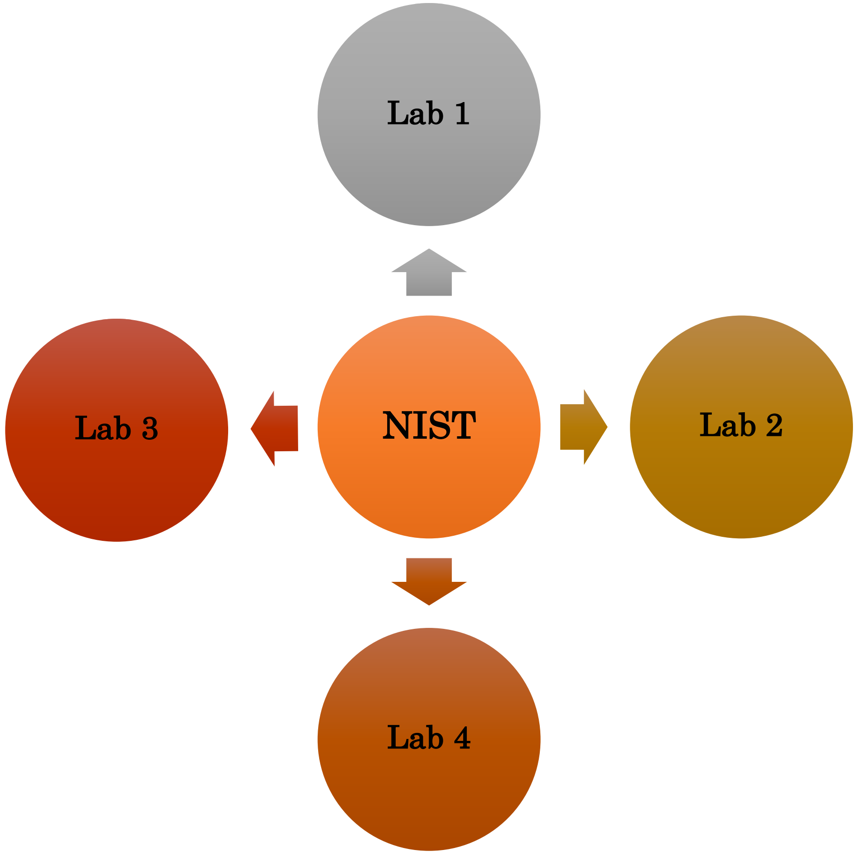
% Relative standard deviation (%RSD)

%RSD = (SD/mean) x 100

How can we measure "accuracy"

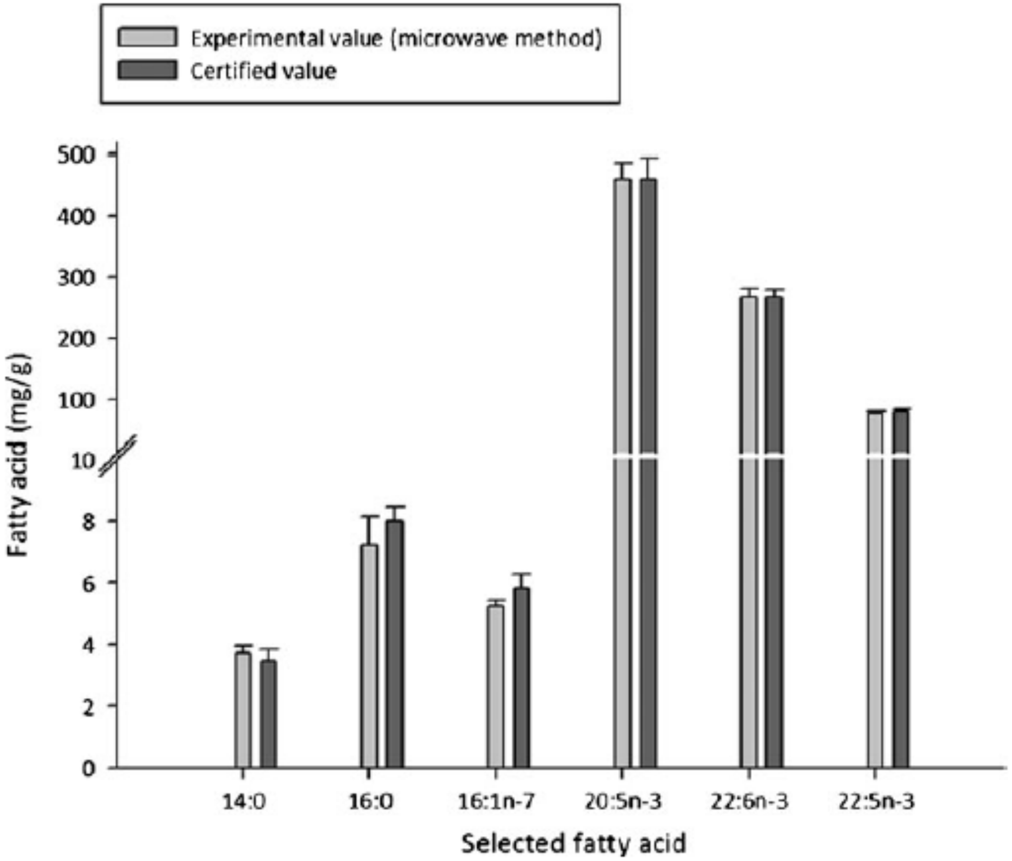
National Institute of Standards and Technology
U.S. Department of Commerce

1. Standard Reference Material (SRM)



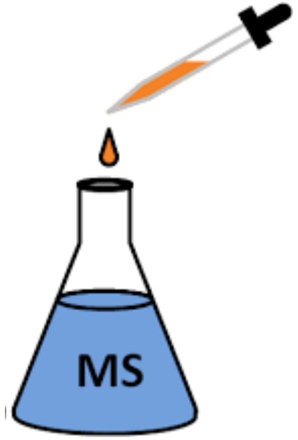
SRM

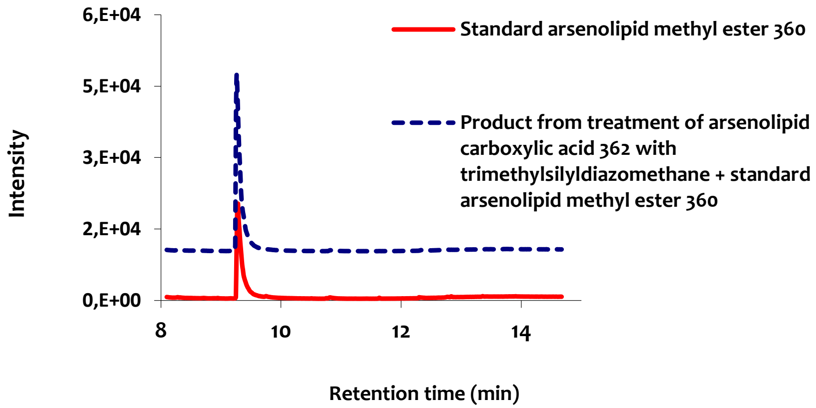
In-house SRM

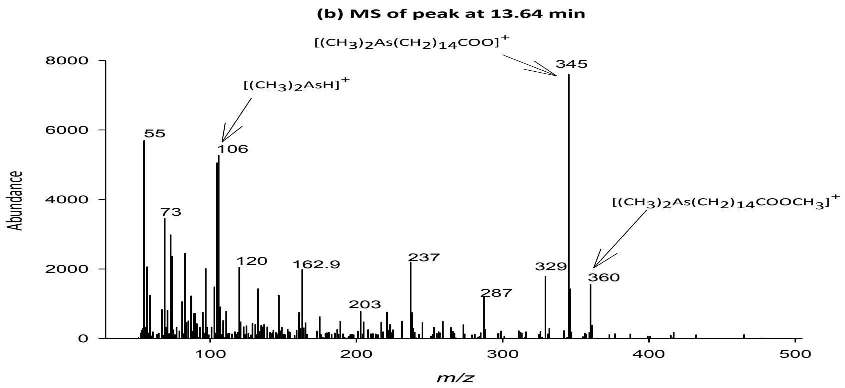


2. Spiking experiment

- % Recovery
- Identity of metabolites





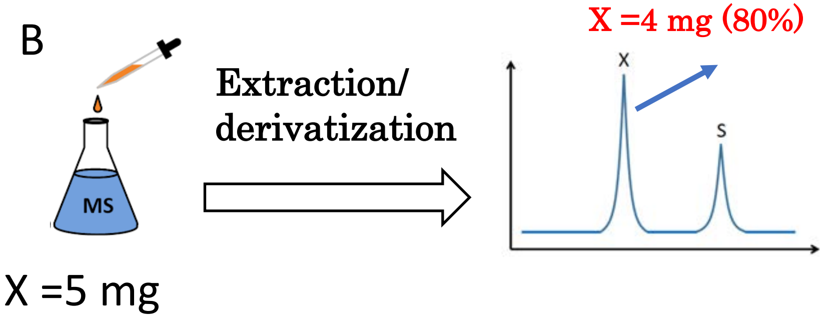


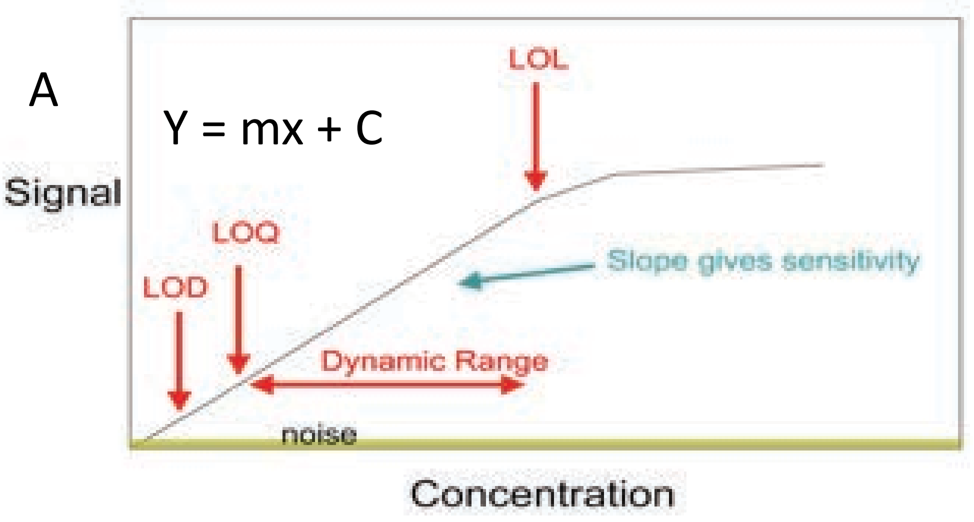
Exp. Conc. (µg As g^{-1})	Measured Conc. (µg As g^{-1})	% Conversion
0.20	0.22 ± 0.02	109 ± 8
0.60	0.66 ± 0.01	110 ± 2
1.11	1.11 ± 0.16	98 ± 14

1. http://unipub.uni-graz.at/obvugrhs/download/pdf/215808?originalFilename=true

1. Quantitative analysis in metabolokics
   -Semi quantification (relative)
   -Absolute quantification

2. Internal standard
3. External standard
4. Standard addition





Metabolomics workflow

Chemical analysis

Sampling → Extraction → Concentration → Analysis

Data mining

Ideas

Inductive Data-driven ↑ ↓ Hypothesis testing

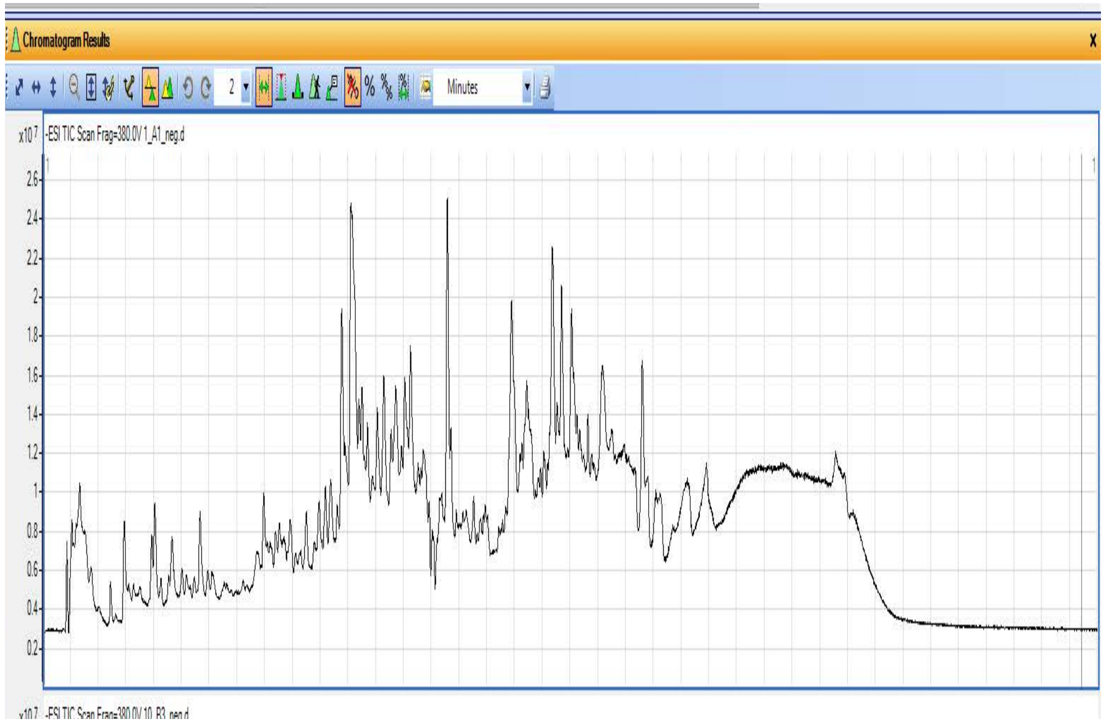
Data

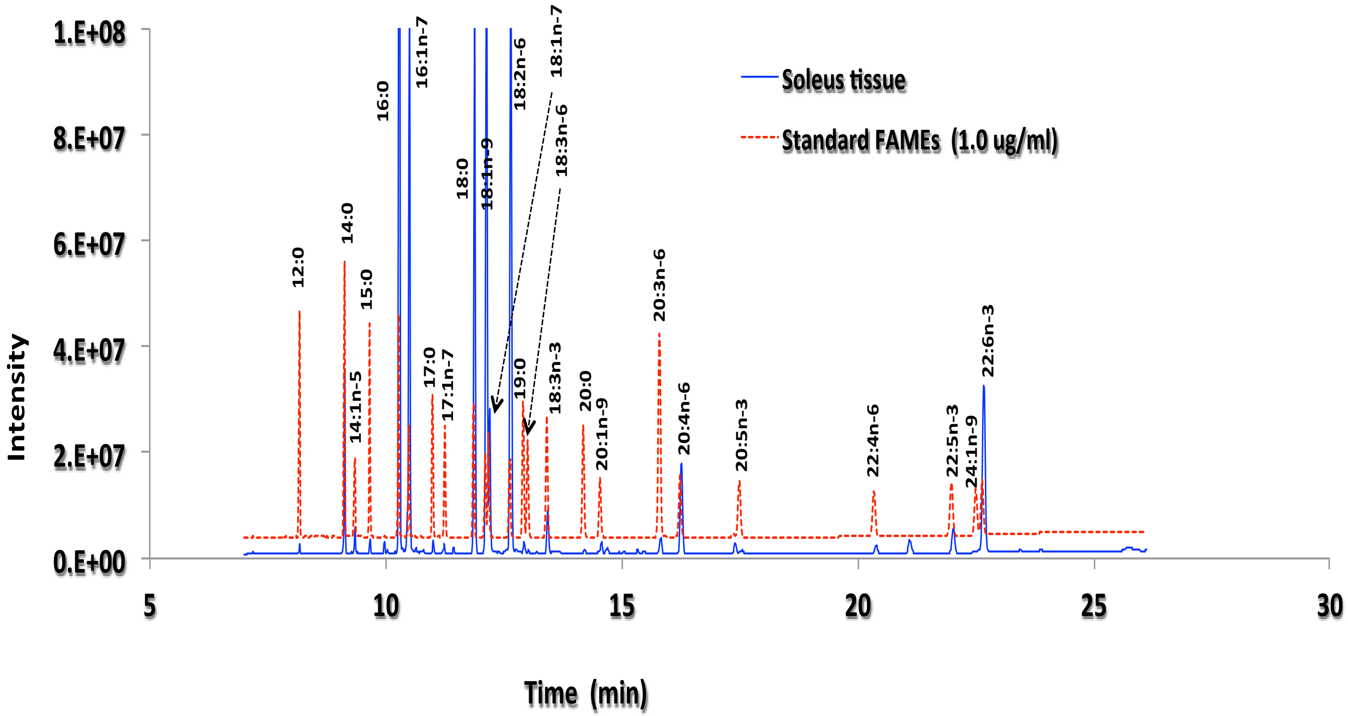
Biochemistry

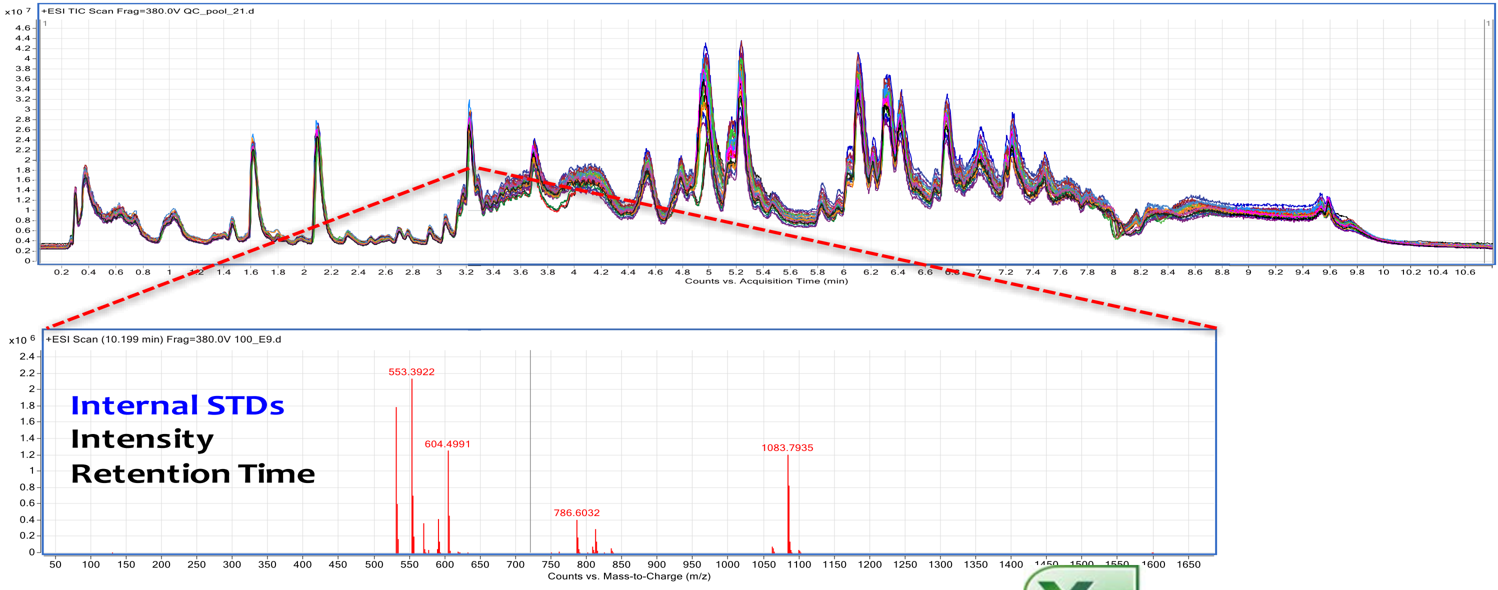
Coupling to biology

Metabolic networks

Data processing (?) and analysis (?)





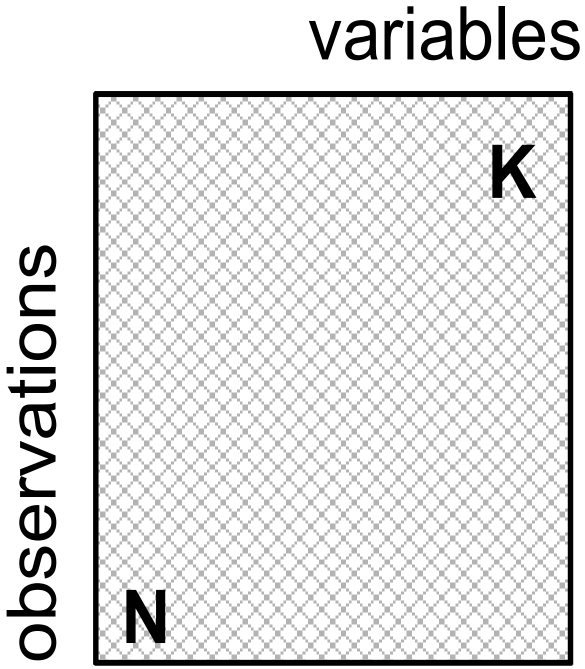


- Internal standards (Area and RT)
- Unknown peaks (Feature: Area and RT)

1600 unidentified metabolites (x 2)

Compound	Blank	Con1	Con_2	Con_3	Con_4	H56_5	TreatM_1	TreaM_2	TreamM_3
785.9907@8.700584	1	747011	2,16E+07	3220174	1738801	2821005	5205011	1888888	2,01E+07
187.0633@2.0974243	1	3,06E+07	3,16E+07	2,85E+07	3,15E+07	3,39E+07	3,46E+07	2,40E+07	2,85E+07
519.3327@6.1120315	16062	7,97E+07	7,43E+07	6,75E+07	6,57E+07	6,49E+07	5,82E+07	7,33E+07	5,57E+07
495.3281@6.316343	42381	7,38E+07	6,14E+07	6,04E+07	5,91E+07	6,05E+07	5,80E+07	6,13E+07	6,03E+07
521.348@6.404964	1	7,50E+07	7,22E+07	7,79E+07	8,05E+07	7,81E+07	6,67E+07	7,52E+07	5,85E+07
523.3635@6.7539687	101894	7,02E+07	6,20E+07	6,94E+07	6,60E+07	6,33E+07	5,66E+07	6,52E+07	5,35E+07

Metabolomics data structure



N Observations

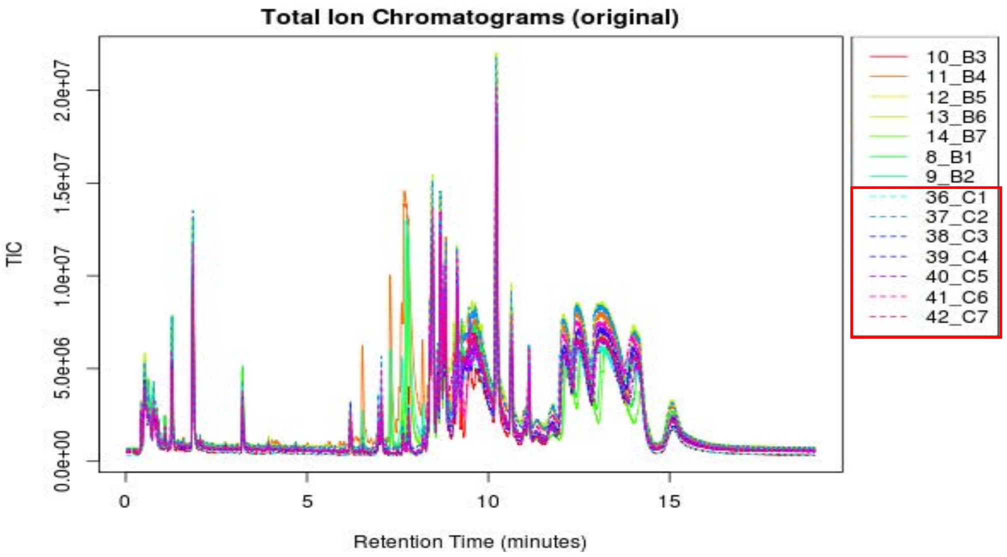
- Analytical samples
- Chemical compounds
- Process time points
- Chemical reactions
- Biological individuals
- Subject individuals

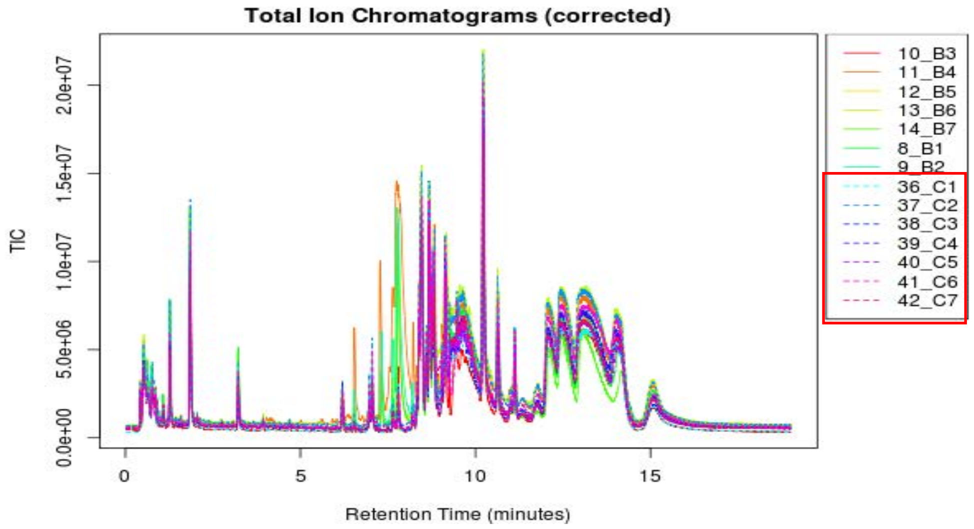
K Variables

- From spectra
  - NMR, IR, UV, MS, ESCA, X-ray, …
- From separation
  - HPLC, GC, TLC, Electrophoresis, Trace elements, …

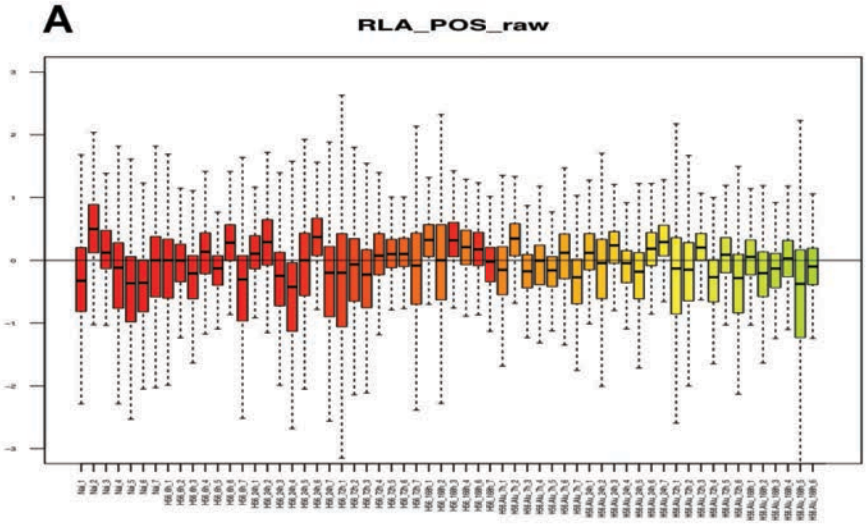
Data processing

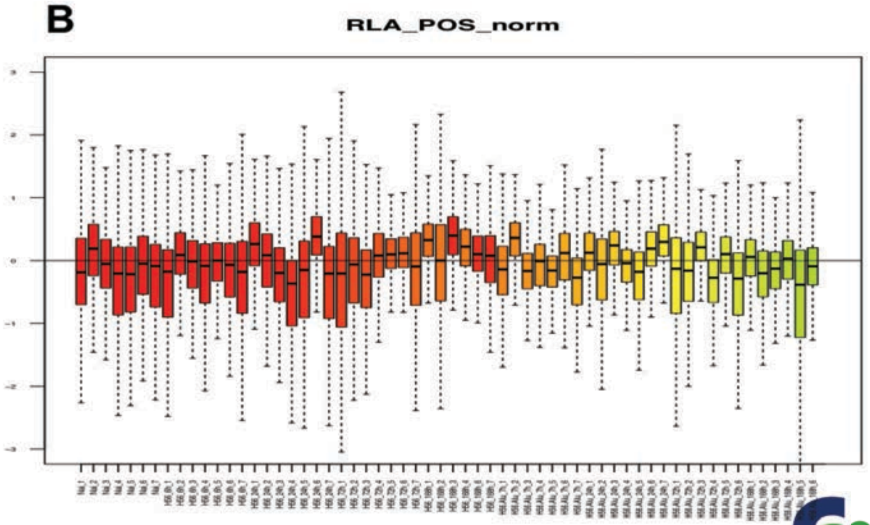
Peak alignment





Normalization





SiMPC Siriraj Metabolomics & Phenomics Center

Data scaling - VARIABLES

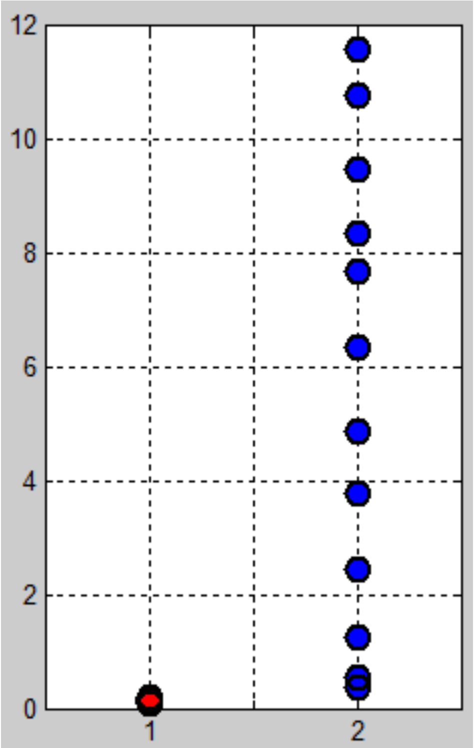
Examples: Center, UV-scaling, Pareto, quantile normalization, Log 2 transformation

- **Problem:** Variables can have substantially different ranges
- **Example:** LOWARP - polymers characterized for strength and warp

Response													
5	6	7	8	9	10	11	12	13	14	15	16	17	18
wrp1	wrp2	wrp3	wrp4	wrp5	wrp6	st1	st2	wrp7	st3	st4	wrp8	st5	st6
0.9	5.0	**0.2**	1.0	0.3	4.2	232	15120	1.2	2190	**26390**	1.3	2400	0.7
3.7	7.3	**0.7**	1.8	2.5	5.4	150	12230	1.8	905	**20270**	2.1	1020	0.6
3.6	6.9	**0.9**	2.1	4.8	9.4	243	15550	1.2	1740	**21180**	1.4	1640	
0.6	3.1	**0.3**	0.4	0.4	1.1	188	11080	1.0	1700	**17630**	1.0	1860	0.5
0.3	2.1	**0.3**	0.3	0.8	1.1	172	11960	1.2	1810	**21070**	1.3	1970	0.5
1.2	5.0					245	15600	1.1	2590	**25310**	1.3	2490	0.6
2.3	3.9	**0.3**	0.4	0.7	1.4	242	13900	1.5	1890	**21370**	1.6	1780	
2.6	5.9	**0.4**	0.2	0.7	1.2	243	17290	1.6	2130	**30530**	1.6	2320	0.7
2.2	5.3	**0.2**	0.7	0.6	2.0	204	11170	1.0	1670	**19070**	1.1	1890	0.6
5.8	7.0	**0.9**	1.0	5.6	11.8	262	20160	1.6	1930	**29830**	1.8	1890	
0.8	2.9	**0.5**	0.6	1.1	2.0	225	14140	1.3	2140	**22850**	1.3	2110	0.7
2.8	5.1	**1.0**	1.2	2.7	6.1	184	15170	1.9	1230	**23400**	2.1	1250	0.6
1.1	4.7	**0.6**	0.9	1.3	3.5	198	13420	1.4	1750	**23790**	1.4	1930	0.7
1.9	4.7	**1.0**	1.0	2.8	5.4	234	16970	1.5	1920	**25010**	1.6	1790	0.7
2.9	5.9	**0.5**	0.6	1.0	6.6	239	15480	1.5	1800	**23140**	1.6	1730	
5.5	7.9	**0.8**	2.4	5.5	9.3	256	18870	1.5	1880	**28440**	1.8	1790	
3.2	6.0	**0.3**	0.5	1.5	5.2	249	16310	1.5	1860	**24710**	1.7	1780	

Data for 2 variables

v1	v2
0.2	0.38
0.21	0.54
0.19	1.23
0.17	2.45
0.18	3.76
0.21	4.87
0.11	6.34
0.14	7.65
0.132	8.32
0.121	9.45
0.14	10.76
0.09	11.55

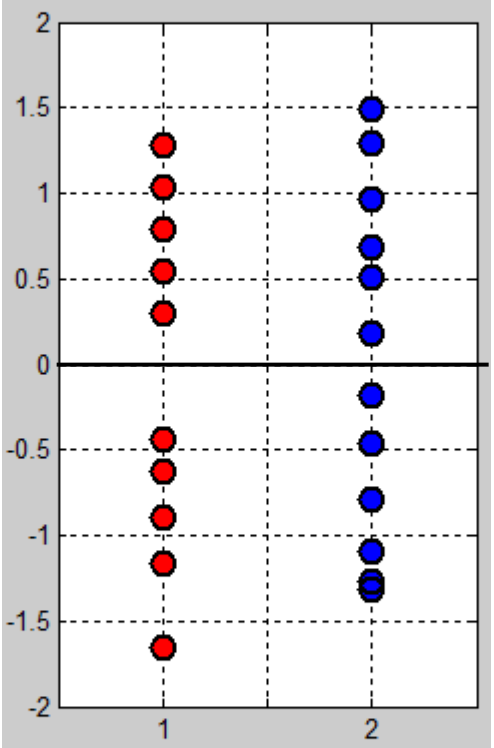


Unscaled - v1 is not easily visualized together with v2

UV-scaling

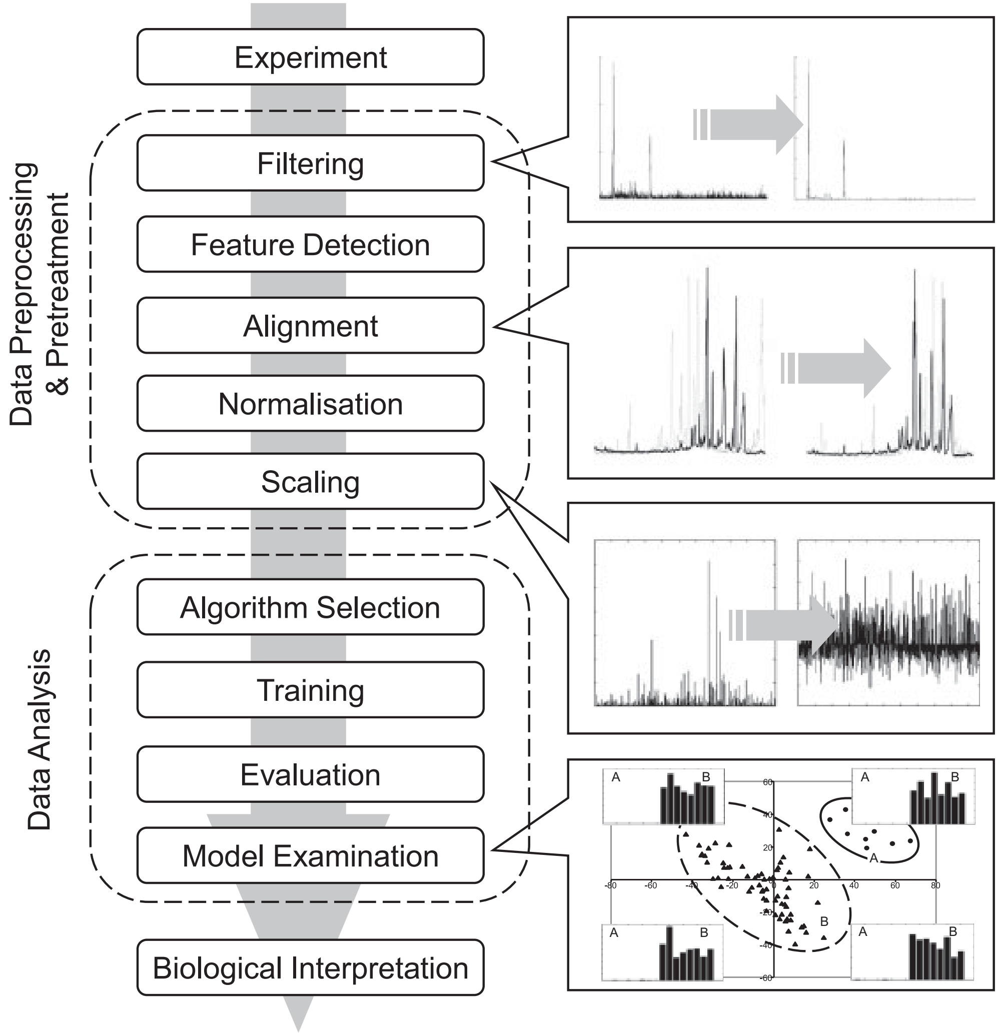
Subtract avg
Divide by stdev

v1 UV	v2 UV
-0.27	-1.34
-0.32	-1.26
-0.27	-1.09
-0.34	-0.78
-0.26	-0.46
-0.27	-0.18
-0.31	0.19
-0.32	0.51
-0.26	0.68
-0.26	0.96
-0.27	1.29
3.17	1.49



Scaled – both variables are more easily understood

1. Automated
2. Unautomated



Reduce analytical noise signal

Proper alignment in both m/z and RT

Scaling is used to adjust importance allocated to the elements of the data In fitting model

[1]Anal. Chem. **(2006)** 78, 779-787 [2] J. Sep. Sci. (2010) 33: 290-304 [3]J. Chromatography A (2007) 1158: 318-328

Data Analysis

1. Univariate analysis

2. Multivariate analysis

Univariate Analysis

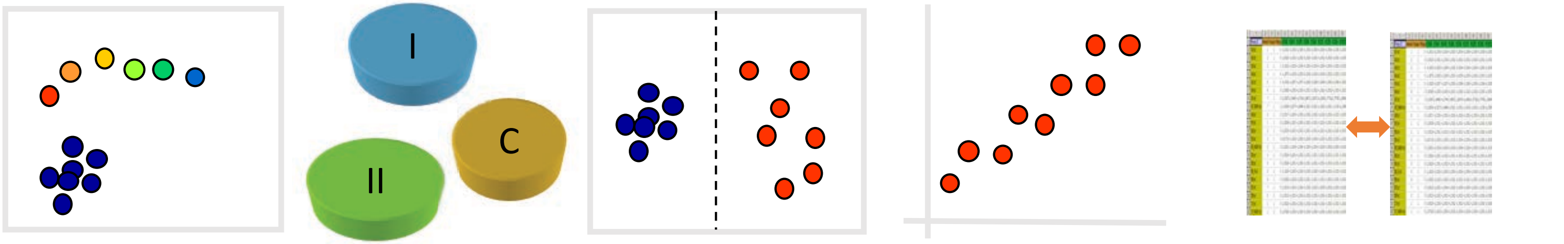
- Univariate means a single variable
- If you measure a population using some single measure such as height, weight, test score, IQ, a metabolite

Met A

P-value (0.05)
Fold change (log2)

Multivariate analysis

"Multivariate statistics is a form of statistics encompassing the simultaneous observation and analysis of more than one outcome variable. The application of multivariate statistics is multivariate analysis"



Overview	Classification	Discrimination	Regression	Multi-block
Trends Outliers Quality Control Biological Diversity Patient Monitoring	Pattern Recognition Diagnostics Healthy/Diseased Toxicity mechanisms Disease progression	Discriminating between groups Biomarker candidates Comparing studies or instrumentation	Prediction methods Biomarker candidates Multivariate calibration QSAR modeling	Understanding and comparing multiple blocks of omics data Metab vs Proteomic vs Genomic
PCA	**SIMCA**	**PLS-DA** **OPLS-DA**	**PLS &OPLS**	**O2PLS & OnPLS**

Other MVA methods
- Multivariate Curve Resolution
- N-way methods (PARAFAC family

Univariate or Multivariate in metabolomics ??

Molecular BioSystems

RSC Publishing

OPINION

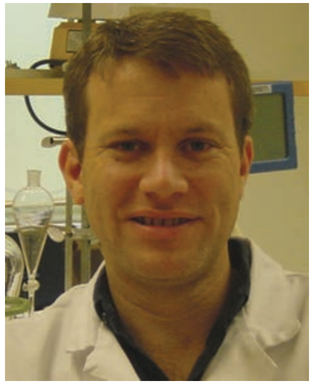
View Article Online
View Journal | View Issue

Trials and tribulations of 'omics data analysis: assessing quality of SIMCA-based multivariate models using examples from pulmonary medicine

Cite this: *Mol. BioSyst.*, 2013, **9**, 2589

sa M. Wheelock*[ab] and Craig E. Wheelock*[bc]

sa M. Wheelock

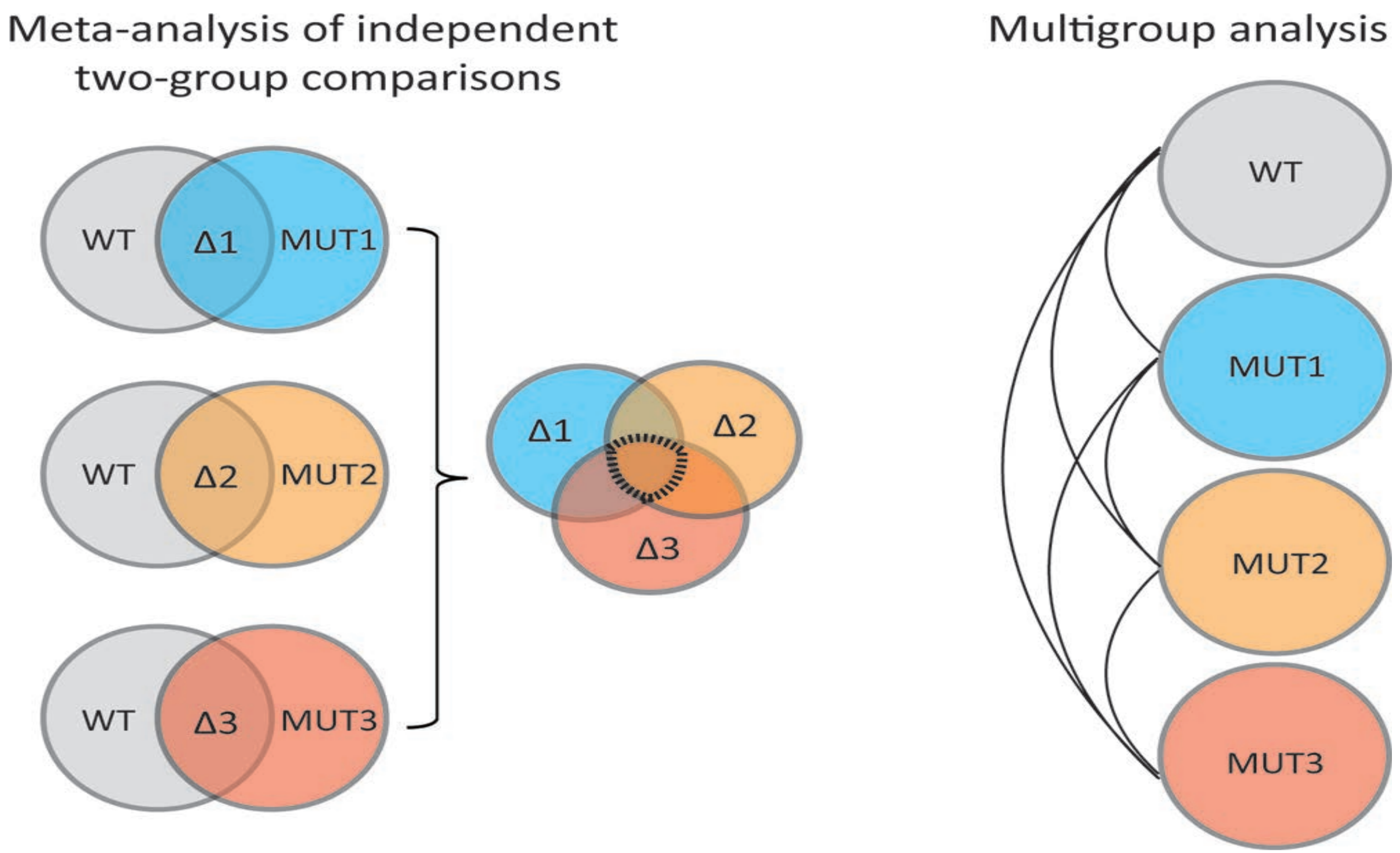


Craig E. Wheelock

Statistical challenges in the analysis of 'omics datasets

The discrepancies between the number of variables analyzed (*e.g.*, mRNA, proteins, metabolites) and the number of study subjects constitutes a major statistical challenge in large-scale omics investigations. These short-and-wide dataset structures are not conducive to traditional univariate statistical methods (*e.g.*, *t*-test), because the repeated hypothesis testing results in a high numbers of potential false positives. The most common way to correct for these high false positive rates is to apply some form of *p*-value correction, such as the False Discovery Rate (FDR) described by Benjamini Hochberg,[12] the *q*-value described by Storey,[13] or Bonferroni correction.[14,15] While the benefits of a reduction of false positives have made these methods the standard in the field for *e.g.*, transcriptomics,[16] the downsides in terms of loss of statistical power to detect true positives are rarely discussed.[17 20] In a previous

Strategy in untargeted analysis

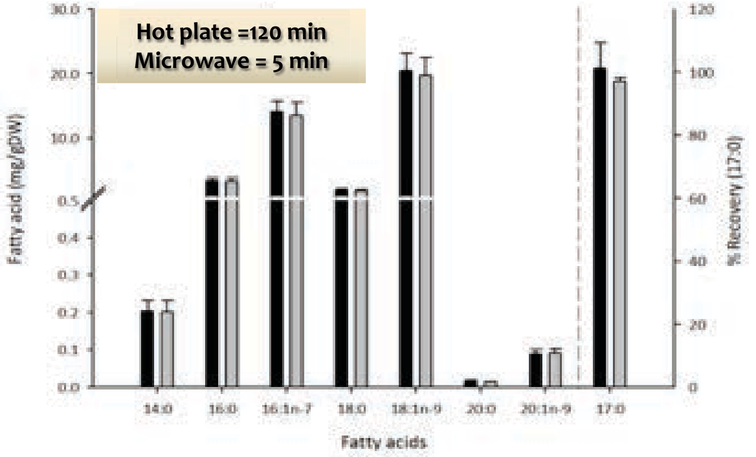


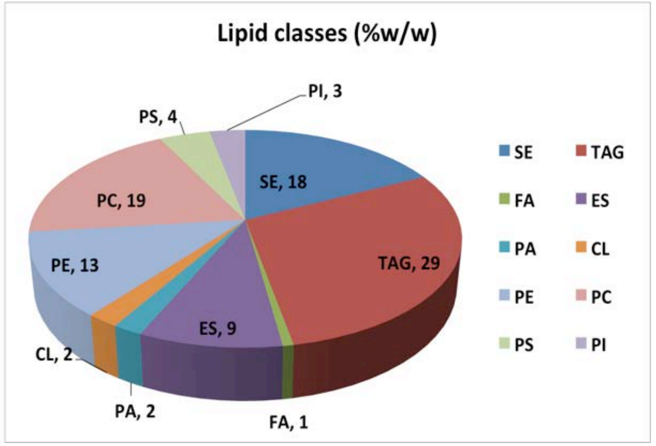
Two-group comparison

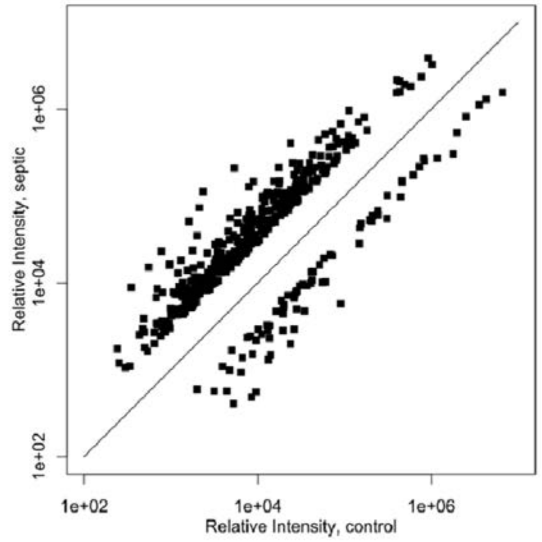
-Unpaired (independent) → different subjects (place and vaccine)

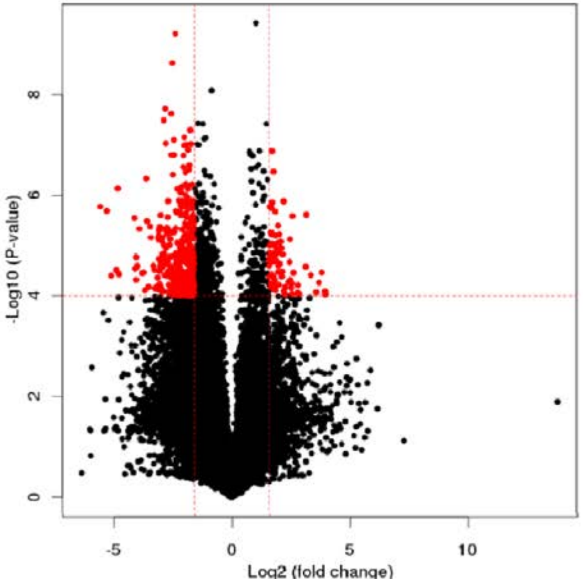
-Paired (dependent) → same subject (before and after)

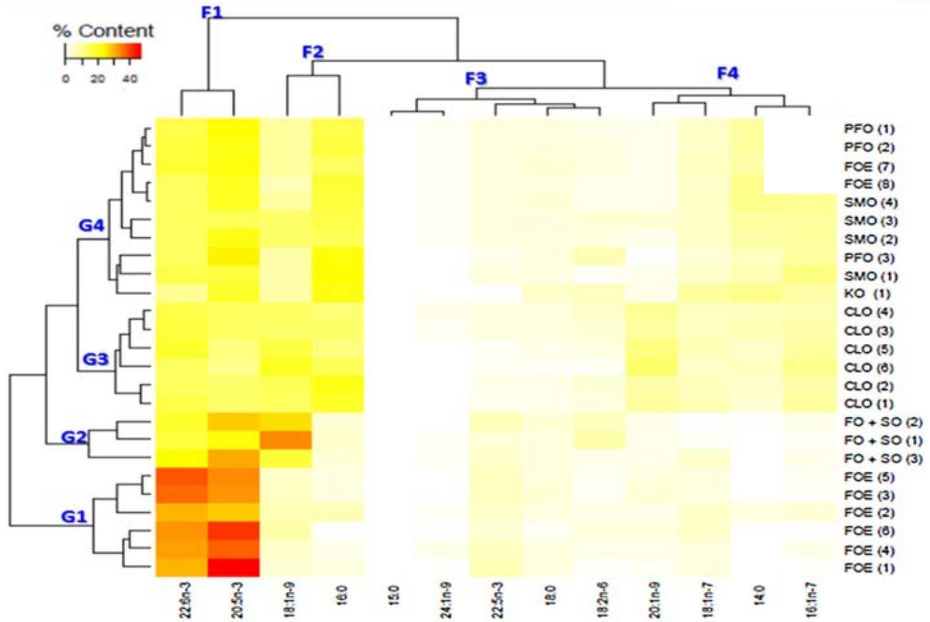
Interpret metabolomics results ?





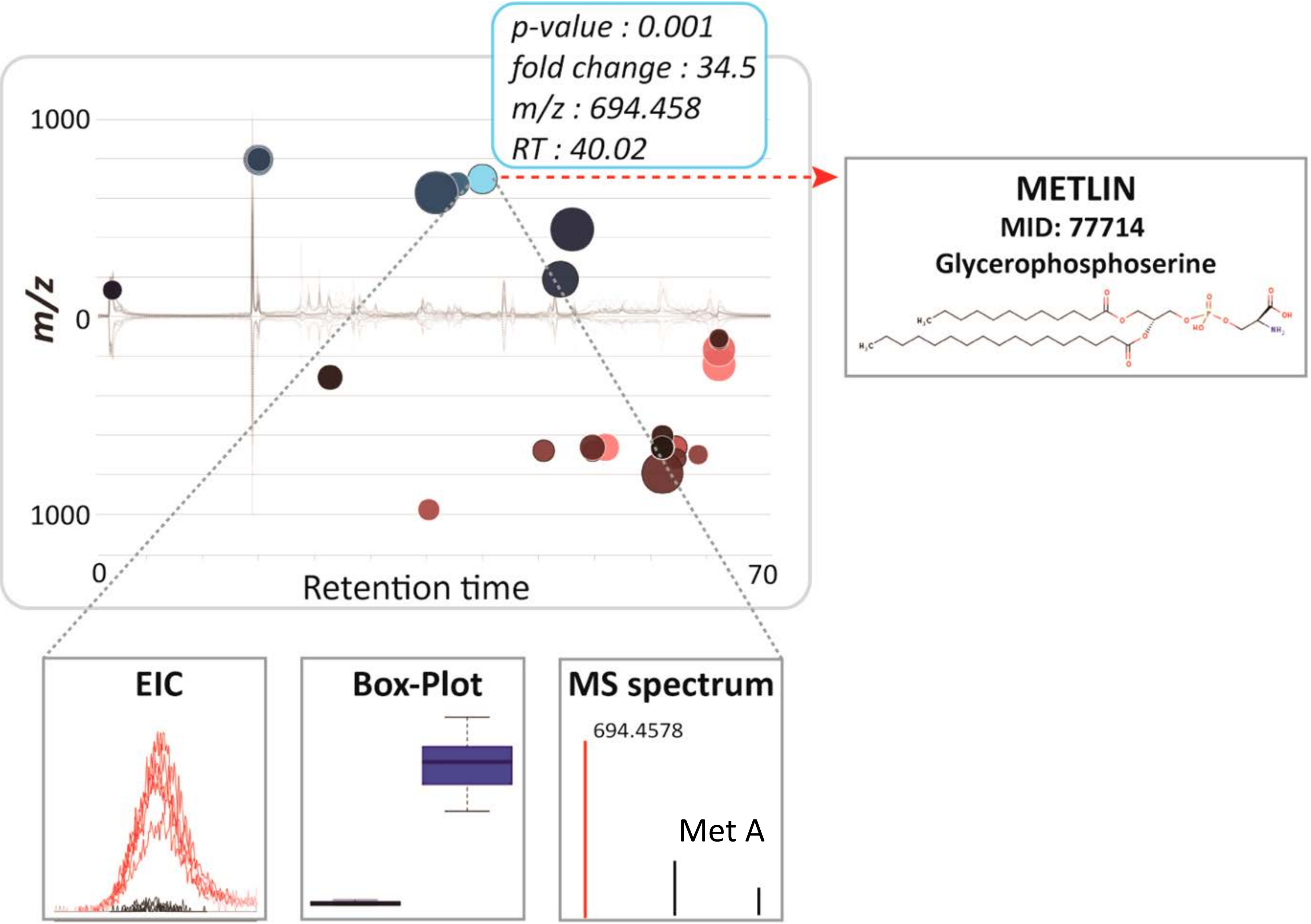






Siriraj Metabolomics & Phenomics Center

Cloud plot

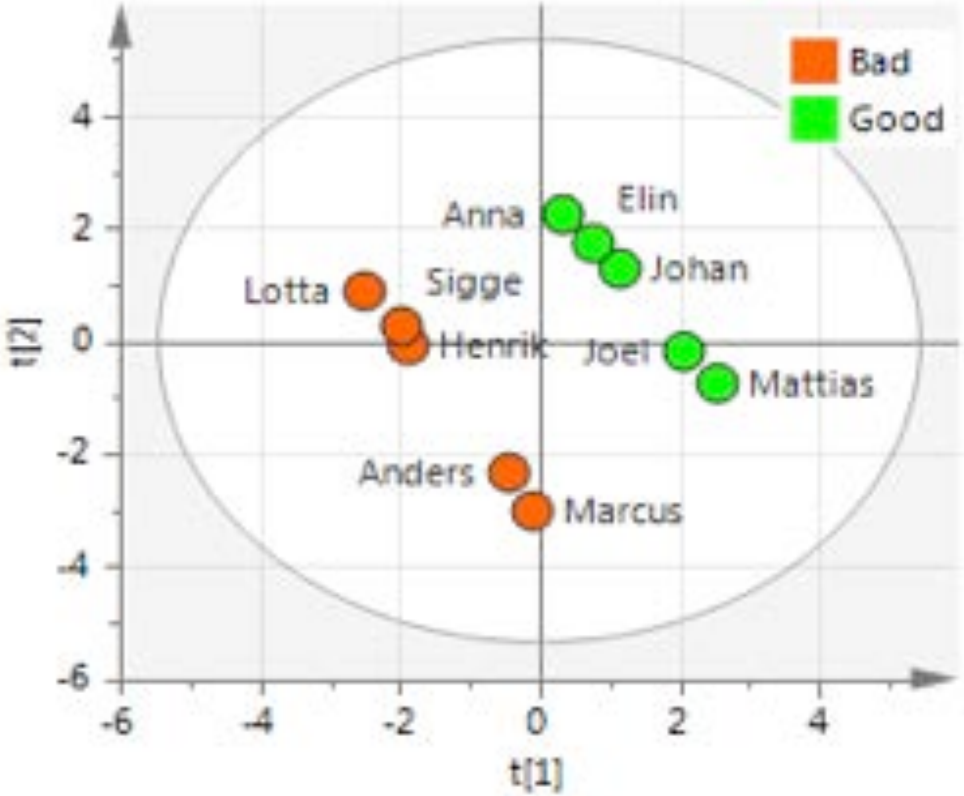


Anal Chem. 2013 15;85(2):798-804

EIC = Extracted Ion Chromatogram

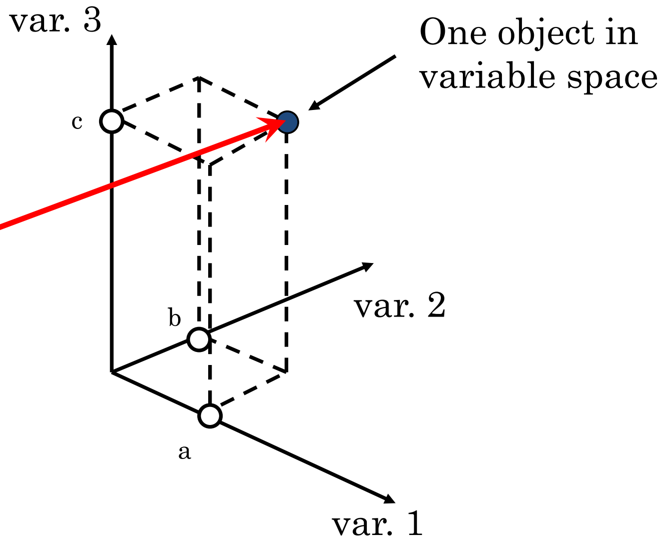
Principal Component Analysis (PCA)

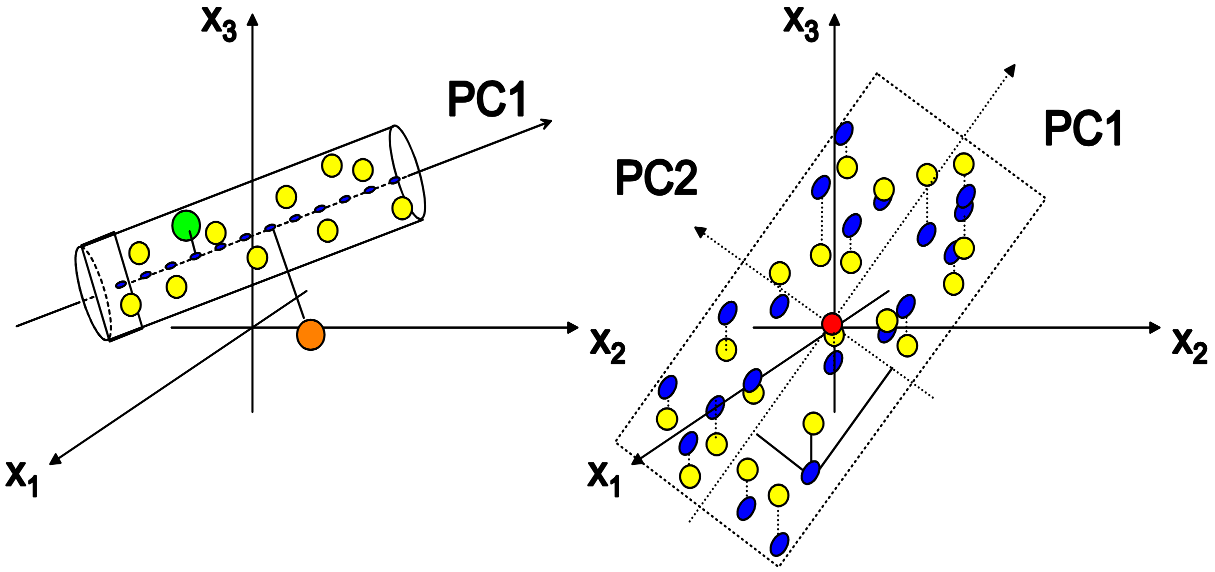
ID / Labels / info					Hours of study/play			Physical variables			??	
Nr	**Name**	**Surname**	**Grades**	**Size**	**Maths**	**Chem**	**Football**	**Height**	**Shoe nr**	**Hand length**	**Surname letters**	**Grades**
1	**Johan**	Frisk	Good	Short	95	84	12	174	40	20.5	5	18.2
2	**Mattias**	Lundin	Good	Tall	92	97	23	191	44	23	6	18
3	**Anna**	Lundell	Good	Short	86	92	21	170	38	18.8	7	16.9
4	**Joel**	Nordlund	Good	Tall	91	87	9	189	43	22	8	17
5	**Elin**	Johansson	Good	Short	90	90	16	172	41	19	9	18.5
6	**Henrik**	Ekman	Bad	Short	49	39	120	168	40	20	5	12.6
7	**Anders**	Holler	Bad	Tall	38	45	112	188	44	22.5	6	12
8	**Sigge**	Hedlund	Bad	Short	45	35	98	173	39	19	7	10.4
9	**Marcus**	Magnuson	Bad	Tall	40	36	105	193	46	23.2	8	10.1
10	**Lotta**	Lundstrom	Bad	Short	35	41	99	170	38	18	9	11.8
							X					**y**



How does PCA work ?

	Var 1	Var 2	Var 3
1			
2			
3	a	b	c
4			
…			
N			



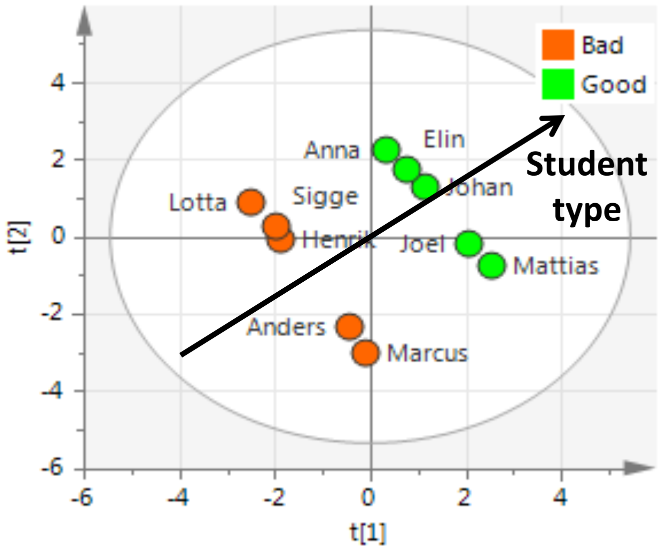


Yellow points are the observed values. Blue points are their PC approximations.

Projected locations on the model (line, plane, or hyperplane) are given by the *scores* (t)

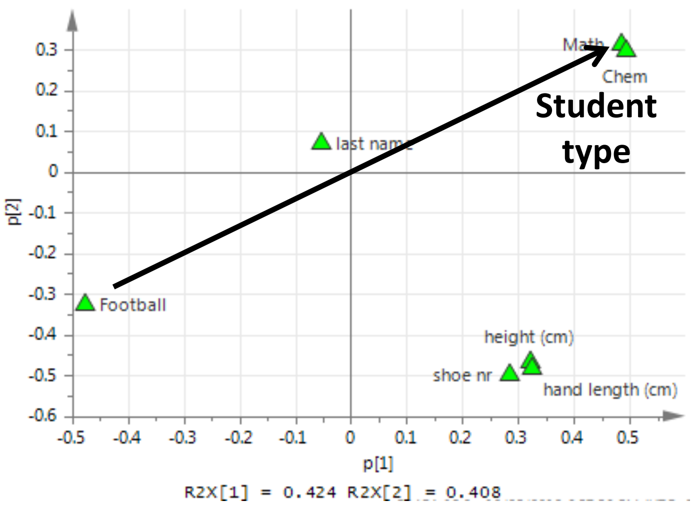
PCA in an unsupervised technique. No assumptions are made about the samples. Adequately labeling the samples (and coloring them) helps us to visualize trends.

Scores



The ”Good” and ”Bad” students are separated

Loadings



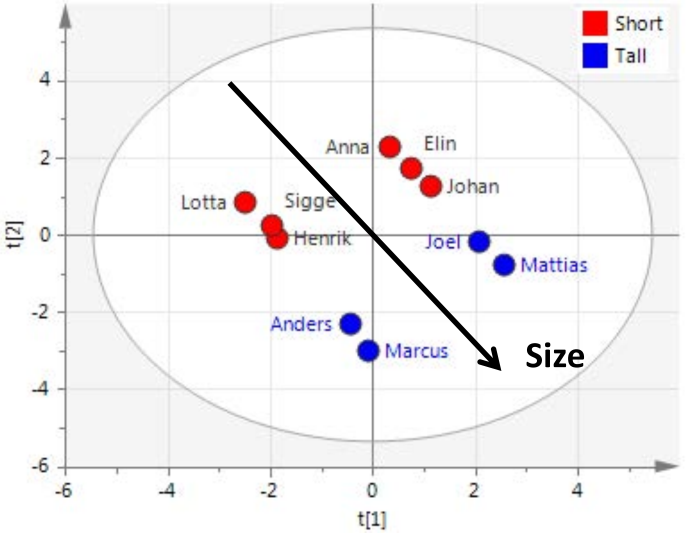
The variables that correlate with each other are observed

The variables that are important in the definition of a ”good” or ”bad” student may be understood from comparing scores and loadings

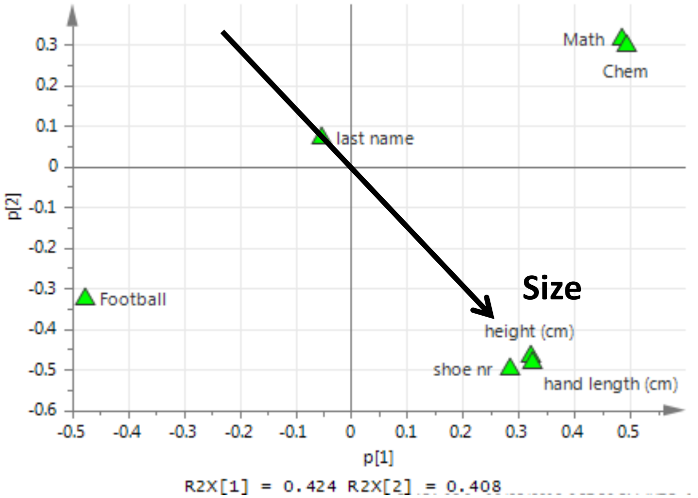
Example PCA students performance (PC1 x PC2)

Now let's color the same plot according to "Short" and "Tall"

Scores



Loadings

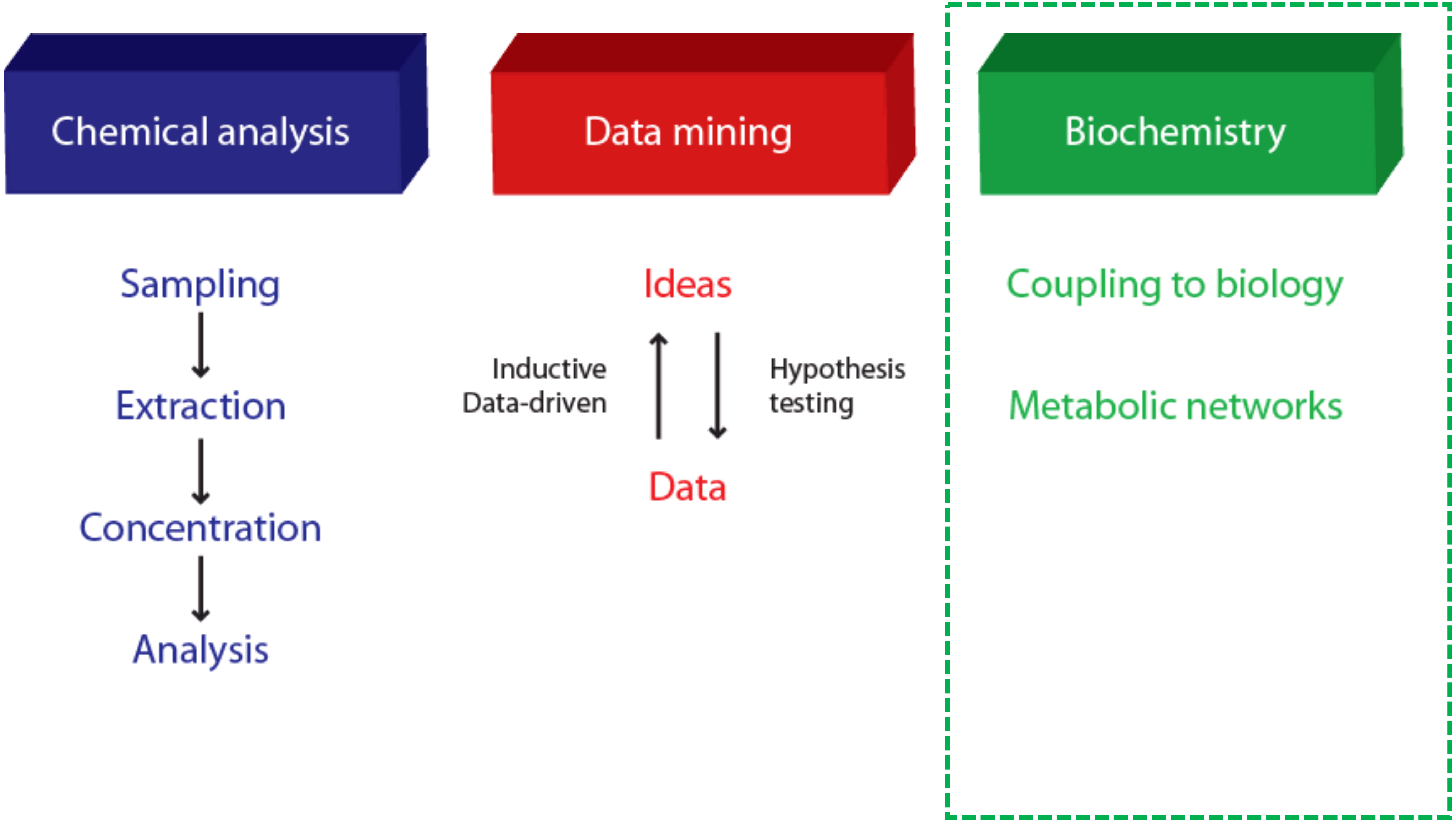


The "Short" and "Tall" students are separated

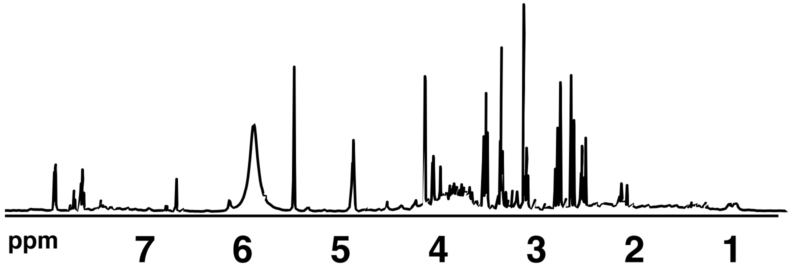
The variables that correlate with each other are observed

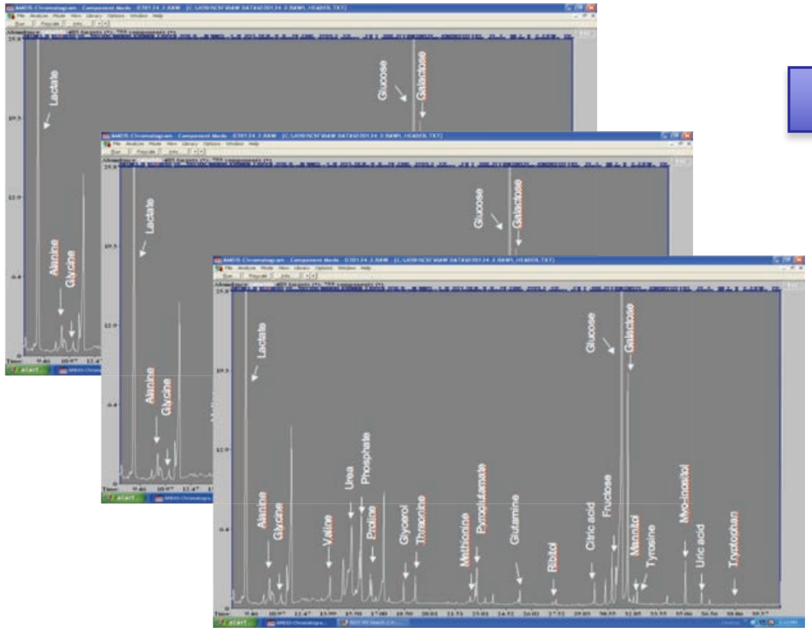
The variables that are important in the definition of a "Short" or "Tall" student may be understood from comparing scores and loadings

Metabolomics workflow



From Spectra to Lists

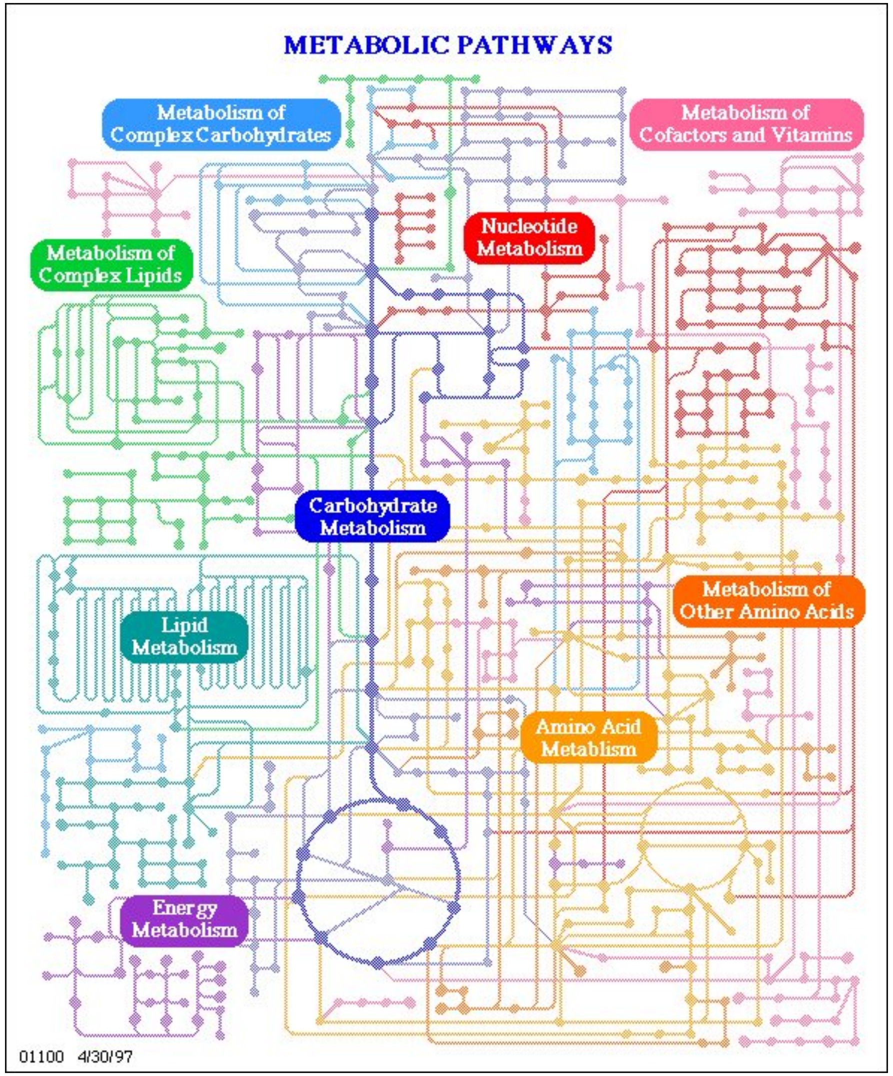




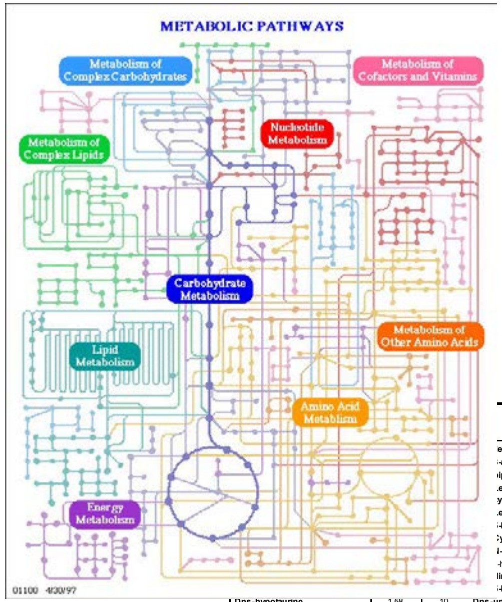
Compound	Retention Time (min)	Conc. in Urine (µM)	Compound	Retention Time (min)	Conc. in Urine (µM)
Dns-o-phospho -L-serine	0.92	<D.L. *	Dns-Ile	6.35	25
Dns-o-phospho -L-tyrosine	0.95	<D.L.	Dns-3-aminosalicylic acid	6.44	0.5
Dns-adnosine monophosphate	0.99	<D.L.	Dns-pipecolic acid	6.50	0.5
Dns-o-phosphoethanolamine	1.06	16	Dns-Leu	6.54	54
Dns-glucosamine	1.06	22	Dns-cystathionine	6.54	0.3
Dns-o-phospho -L-threonine	1.09	<D.L.	Dns-Leu-Pro	6.60	0.4
Dns-6-dimet hylamine purine	1.20	<D.L.	Dns-5-hydroxylysine	6.65	1.6
Dns-3-methyl -histidine	1.22	80	Dns-Cystine	6.73	160
Dns-taurine	1.25	834	Dns-N-norleucine	6.81	0.1
Dns-carnosine	1.34	28	Dns-5-hydroxydopamine	7.17	<D.L.
Dns-Arg	1.53	36	Dns-dimethylamine	7.33	293
Dns-Asn	1.55	133	Dns-5-HIAA	7.46	18
Dns-hypotaurine	1.58	10	Dns-umbelliferone	7.47	1.9
Dns-homocarnosine	1.61	3.9	Dns-2,3 -diaminoproprionic acid	7.63	<D.L.
Dns-guanidine	1.62	<D.L.	Dns-L-ornithine	7.70	15
Dns-Gln	1.72	633	Dns-4-acetyamidophenol	7.73	51
Dns-allantoin	1.83	3.8	Dns-procaine	7.73	8.9
Dns-L-citrulline	1.87	2.9	Dns-homocystine	7.76	3.3
Dns-1 (or 3 -)-methylhistamine	1.94	1.9	Dns-acetaminophen	7.97	82
Dns-adenosine	2.06	2.6	Dns-Phe-Phe	8.03	0.4
Dns-methylguanidine	2.20	<D.L.	Dns-5-methyo xysalicylic acid	8.04	2.1
Dns-Ser	2.24	511	Dns-Lys	8.16	184
Dns-aspartic acid amide	2.44	26	Dns-aniline	8.17	<D.L.
Dns-4-hydroxy -proline	2.56	2.3	Dns-leu-Phe	8.22	0.3
Dns-Glu	2.57	21	Dns-His	8.35	1550
Dns-Asp	2.60	90	Dns-4-thialysine	8.37	<D.L.
Dns-Thr	3.03	157	Dns-benzylamine	8.38	<D.L.
Dns-epinephrine	3.05	<D.L.	Dns-1-ephedrine	8.50	0.6
Dns-ethanolamine	3.11	471	Dns-tryptamine	8.63	0.4
Dns-aminoadipic acid	3.17	70	Dns-pyrydoxamine	8.94	<D.L.
Dns-Gly	3.43	2510	Dns-2-methyl -benzylamine	9.24	<D.L.
Dns-Ala	3.88	593	Dns-5-hydroxytrptophan	9.25	0.12
Dns-aminolevulinic acid	3.97	30	Dns-1,3 -diaminopropane	9.44	0.23
Dns-r-amino-butyric acid	3.98	4.6	Dns-putrescine	9.60	0.5
Dns-p-amino-hippuric acid	3.98	2.9	Dns-1,2 -diaminopropane	9.66	0.1
Dns-5-hydro xymethyluricil	4.58	1.9	Dns-tyrosinamide	9.79	29
Dns-tryptophanamide	4.70	5.5	Dns-dopamine	10.08	140
Dns-isoguanine	4.75	<D.L.	Dns-cadaverine	10.08	0.08
Dns-5-aminopentanoic acid	4.79	1.6	Dns-histamine	10.19	0.4
Dns-sarcosine	4.81	7.2	Dns-3-methoxy -tyramine	10.19	9.2
Dns-3-amino -isobutyrate	4.81	85	Dns-Tyr	10.28	321
Dns-2-aminobutyric acid	4.91	17	Dns-cysteamine	10.44	<D.L.

From Lists to Pathways

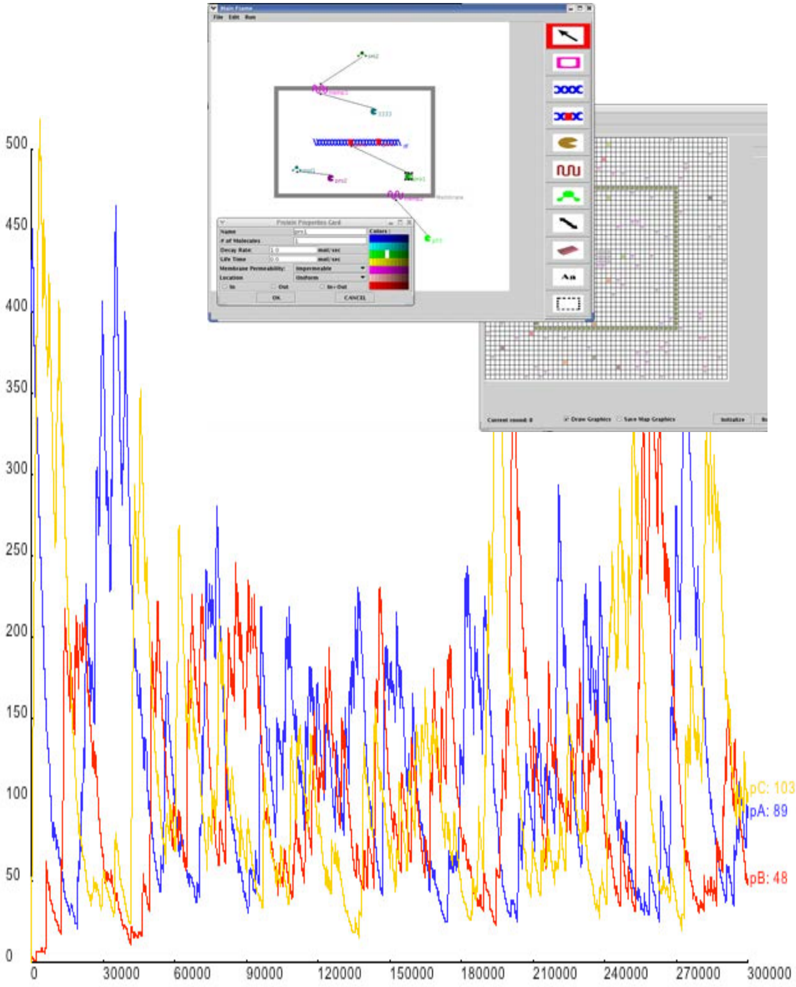
Compound	Retention Time (min)	Conc. in Urine (µM)	Compound	Retention Time (min)	Conc. in Urine (µM)
Dns-o-phospho -L-serine	0.92	<D.L. *	Dns-Ile	6.35	25
Dns-o-phospho -L-tyrosine	0.95	<D.L.	Dns-3-aminosalicylic acid	6.44	0.5
Dns-adnosine monophosphate	0.99	<D.L.	Dns-pipecolic acid	6.50	0.5
Dns-o-phosphoethanolamine	1.06	16	Dns-Leu	6.54	54
Dns-glucosamine	1.06	22	Dns-cystathionine	6.54	0.3
Dns-o-phospho -L-threonine	1.09	<D.L.	Dns-Leu-Pro	6.60	0.4
Dns-6-dimet hylamine purine	1.20	<D.L.	Dns-5-hydroxylysine	6.65	1.6
Dns-3-methyl -histidine	1.22	80	Dns-Cystine	6.73	160
Dns-taurine	1.25	834	Dns-N-norleucine	6.81	0.1
Dns-carnosine	1.34	28	Dns-5-hydroxydopamine	7.17	<D.L.
Dns-Arg	1.53	36	Dns-dimethylamine	7.33	293
Dns-Asn	1.55	133	Dns-5-HIAA	7.46	18
Dns-hypotaurine	1.58	10	Dns-umbelliferone	7.47	1.9
Dns-homocarnosine	1.61	3.9	Dns-2,3 -diaminoproprionic acid	7.63	<D.L.
Dns-guanidine	1.62	<D.L.	Dns-L-ornithine	7.70	15
Dns-Gln	1.72	633	Dns-4-acetyamidophenol	7.73	51
Dns-allantoin	1.83	3.8	Dns-procaine	7.73	8.9
Dns-L-citrulline	1.87	2.9	Dns-homocystine	7.76	3.3
Dns-1 (or 3 -)-methylhistamine	1.94	1.9	Dns-acetaminophen	7.97	82
Dns-adenosine	2.06	2.6	Dns-Phe-Phe	8.03	0.4
Dns-methylguanidine	2.20	<D.L.	Dns-5-methyo xysalicylic acid	8.04	2.1
Dns-Ser	2.24	511	Dns-Lys	8.16	184
Dns-aspartic acid amide	2.44	26	Dns-aniline	8.17	<D.L.
Dns-4-hydroxy -proline	2.56	2.3	Dns-leu-Phe	8.22	0.3
Dns-Glu	2.57	21	Dns-His	8.35	1550
Dns-Asp	2.60	90	Dns-4-thialysine	8.37	<D.L.
Dns-Thr	3.03	157	Dns-benzylamine	8.38	<D.L.
Dns-epinephrine	3.05	<D.L.	Dns-1-ephedrine	8.50	0.6
Dns-ethanolamine	3.11	471	Dns-tryptamine	8.63	0.4
Dns-aminoadipic acid	3.17	70	Dns-pyrydoxamine	8.94	<D.L.
Dns-Gly	3.43	2510	Dns-2-methyl -benzylamine	9.24	<D.L.
Dns-Ala	3.88	593	Dns-5-hydroxytrptophan	9.25	0.12
Dns-aminolevulinic acid	3.97	30	Dns-1,3 -diaminopropane	9.44	0.23
Dns-r-amino-butyric acid	3.98	4.6	Dns-putrescine	9.60	0.5
Dns-p-amino-hippuric acid	3.98	2.9	Dns-1,2 -diaminopropane	9.66	0.1
Dns-5-hydro xymethyluricil	4.58	1.9	Dns-tyrosinamide	9.79	29
Dns-tryptophanamide	4.70	5.5	Dns-dopamine	10.08	140
Dns-isoguanine	4.75	<D.L.	Dns-cadaverine	10.08	0.08
Dns-5-aminopentanoic acid	4.79	1.6	Dns-histamine	10.19	0.4
Dns-sarcosine	4.81	7.2	Dns-3-methoxy -tyramine	10.19	9.2
Dns-3-amino -isobutyrate	4.81	85	Dns-Tyr	10.28	321
Dns-2-aminobutyric acid	4.91	17	Dns-cysteamine	10.44	<D.L.



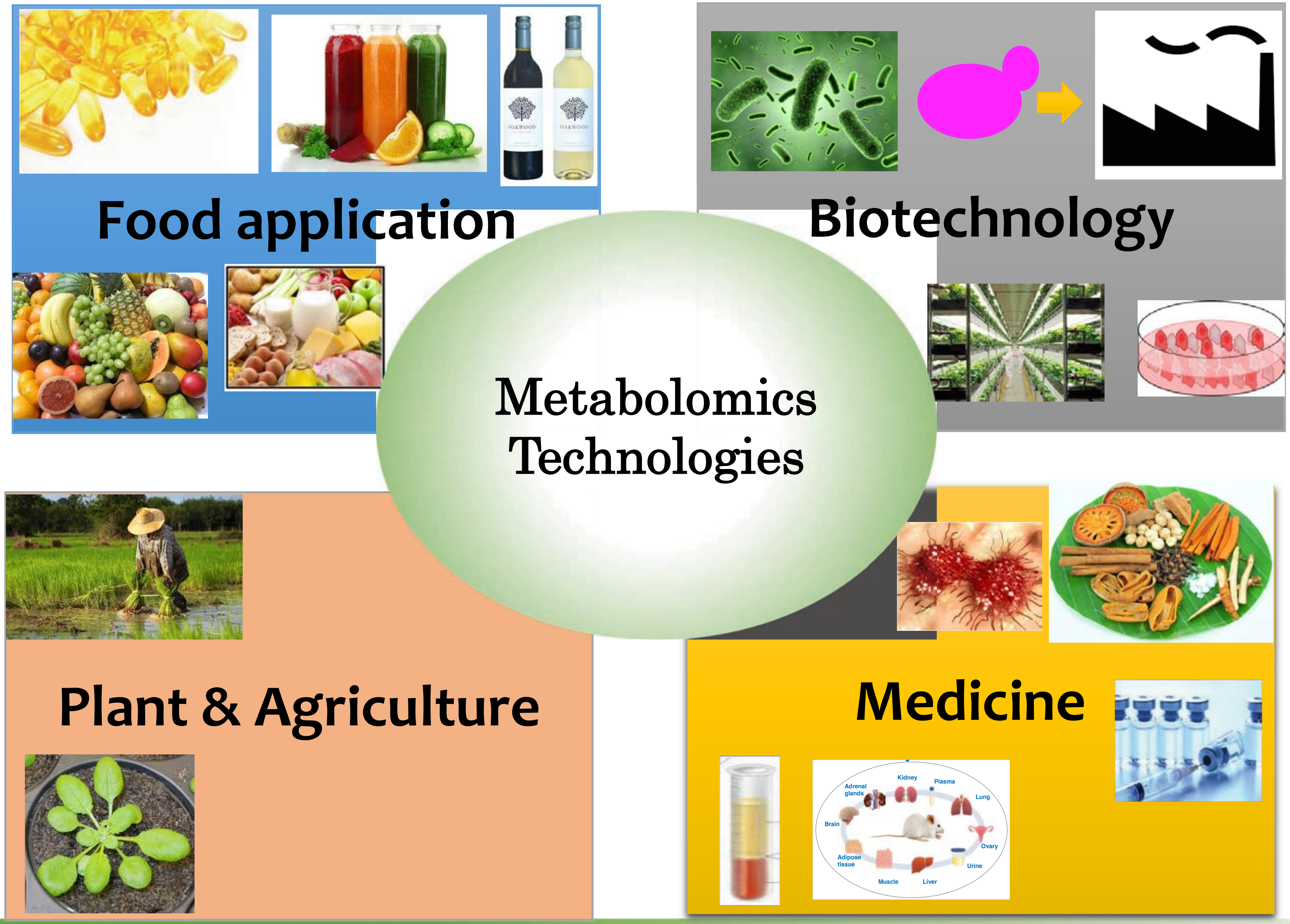
From Pathways & Lists to Models & Biomarkers



			Compound	Retention Time (min)	Conc. in Urine (μM)
			e	6.35	25
			-aminosalicylic acid	6.44	0.5
			ipecolic acid	6.50	0.5
			.eu	6.54	54
			ystathionine	6.54	0.3
			.eu -Pro	6.60	0.4
			-hydroxylysine	6.65	1.6
			ystine	6.73	160
			-norleucine	6.81	0.1
			-hydroxydopamine	7.17	<D.L.
			limethylamine	7.33	293
			-HIAA	7.46	18
Dns-hypotaurine	1.58	10	Dns-umbelliferone	7.47	1.9
Dns-homocarnosine	1.61	3.9	Dns-2,3 -diaminoproprionic acid	7.63	<D.L.
Dns-guanidine	1.62	<D.L.	Dns-L-ornithine	7.70	15
Dns-Gln	1.72	633	Dns-4-acetyamidophenol	7.73	51
Dns-allantoin	1.83	3.8	Dns-procaine	7.73	8.9
Dns-L-citrulline	1.87	2.9	Dns-homocystine	7.76	3.3
Dns-1 (or 3 -)-methylhistamine	1.94	1.9	Dns-acetaminophen	7.97	82
Dns-adenosine	2.06	2.6	Dns-Phe-Phe	8.03	0.4
Dns-methylguanidine	2.20	<D.L.	Dns-5-methyo xysalicylic acid	8.04	2.1
Dns-Ser	2.24	511	Dns-Lys	8.16	184
Dns-aspartic acid amide	2.44	26	Dns-aniline	8.17	<D.L.
Dns-4-hydroxy -proline	2.56	2.3	Dns-leu -Phe	8.22	0.3
Dns-Glu	2.57	21	Dns-His	8.35	1550
Dns-Asp	2.60	90	Dns-4-thialysine	8.37	<D.L.
Dns-Thr	3.03	157	Dns-benzylamine	8.38	<D.L.
Dns-epinephrine	3.05	<D.L.	Dns-1-ephedrine	8.50	0.6
Dns-ethanolamine	3.11	471	Dns-tryptamine	8.63	0.4
Dns-aminoadipic acid	3.17	70	Dns-pyrydoxamine	8.94	<D.L.
Dns-Gly	3.43	2510	Dns-2-methyl -benzylamine	9.24	<D.L.
Dns-Ala	3.88	593	Dns-5-hydroxytrptophan	9.25	0.12
Dns-aminolevulinic acid	3.97	30	Dns-1,3 -diaminopropane	9.44	0.23
Dns-r-amino -butyric acid	3.98	4.6	Dns-putrescine	9.60	0.5
Dns-p-amino -hippuric acid	3.98	2.9	Dns-1,2 -diaminopropane	9.66	0.1
Dns-5-hydro xymethyluricil	4.58	1.9	Dns-tyrosinamide	9.79	29
Dns-tryptophanamide	4.70	5.5	Dns-dopamine	10.08	140
Dns-isoguanine	4.75	<D.L.	Dns-cadaverine	10.08	0.08
Dns-5-aminopentanoic acid	4.79	1.6	Dns-histamine	10.19	0.4
Dns-sarcosine	4.81	7.2	Dns-3-methoxy -tyramine	10.19	9.2
Dns-3-amino -isobutyrate	4.81	85	Dns-Tyr	10.28	321
Dns-2-aminobutyric acid	4.91	17	Dns-cysteamine	10.44	<D.L.



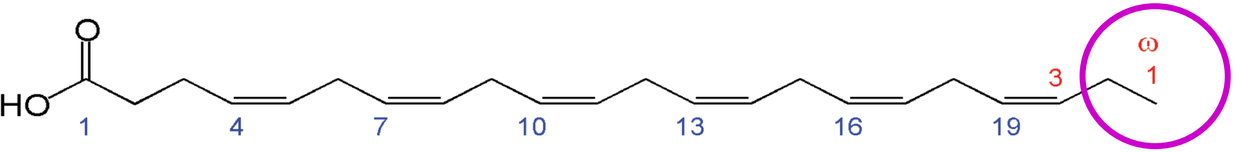
Metabolomics application in life sciences



Example I
Food

Greenland Eskimos

Dyerberg at al (1978)



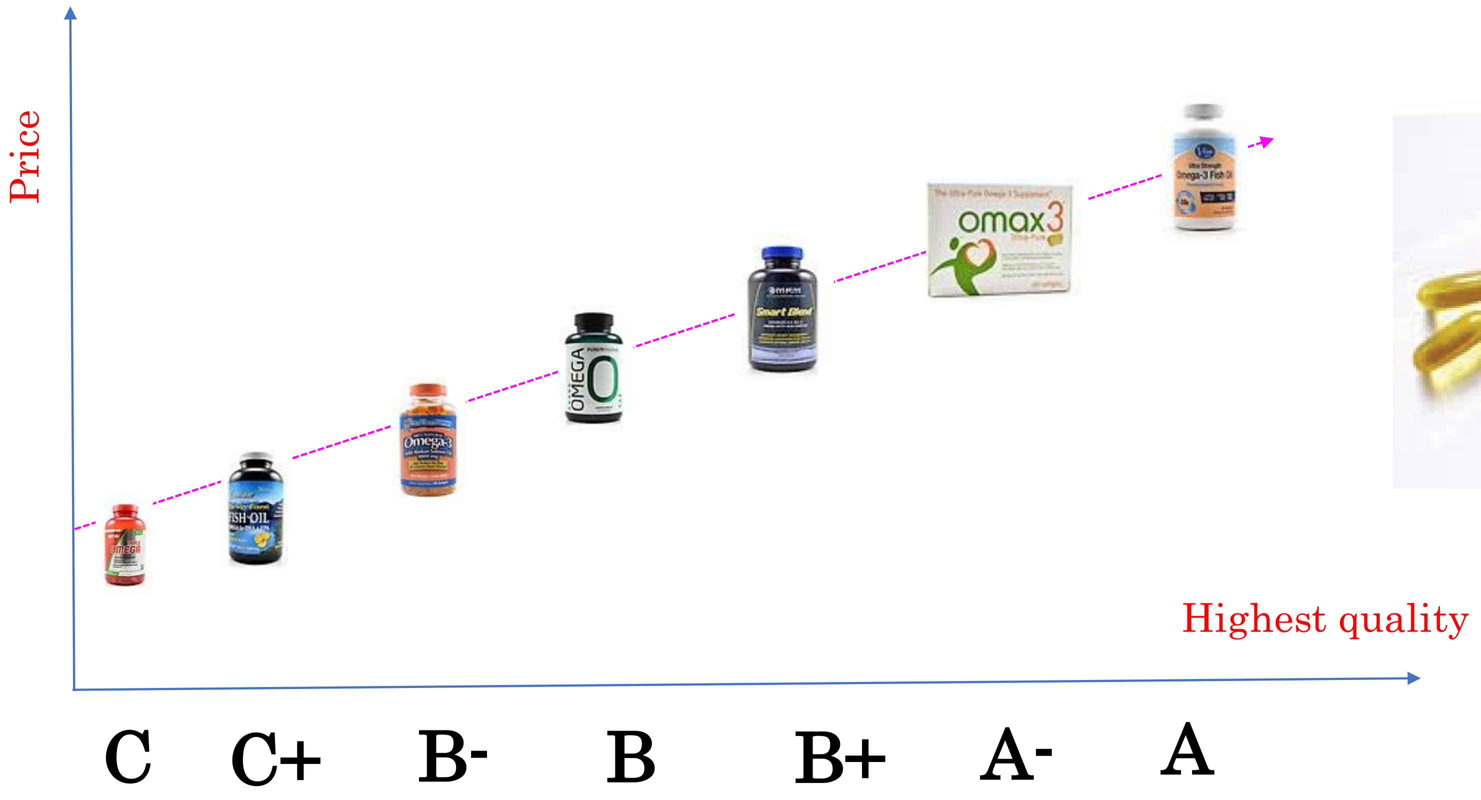
Unsat. FA

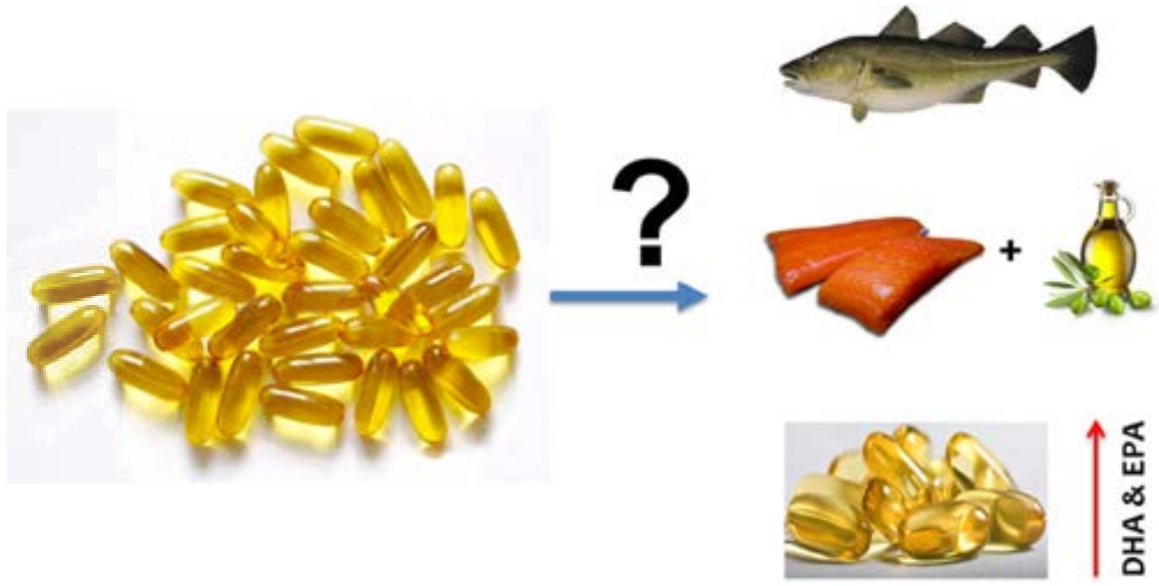
22:6 n-3

Dyerberg et al., (1978)The lancet 312 : 117-119

Research Question

Type/ grade of fish oil supplements



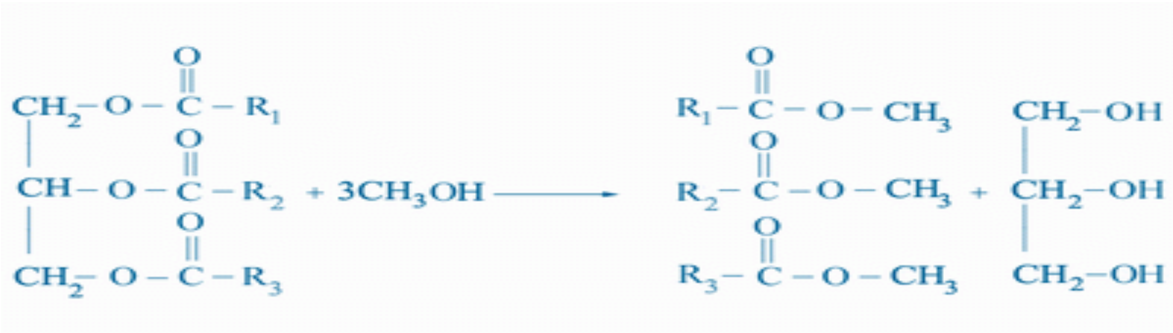


Khoomrung et al. (2014) Eur. Lipid. Sci. Tech. 116:795-804

Fish oil supplement samples

Group	Tpye of fish oil	Number of sample	Code
1	Fish oil enriched	8	FOE
2	Pure fish oil	3	PFO
3	Salmon oil	4	SMO
4	Cod liver oil	6	CLO
5	Krill oil	1	KO
6	Fish oil + plant oil	3	FO+SO

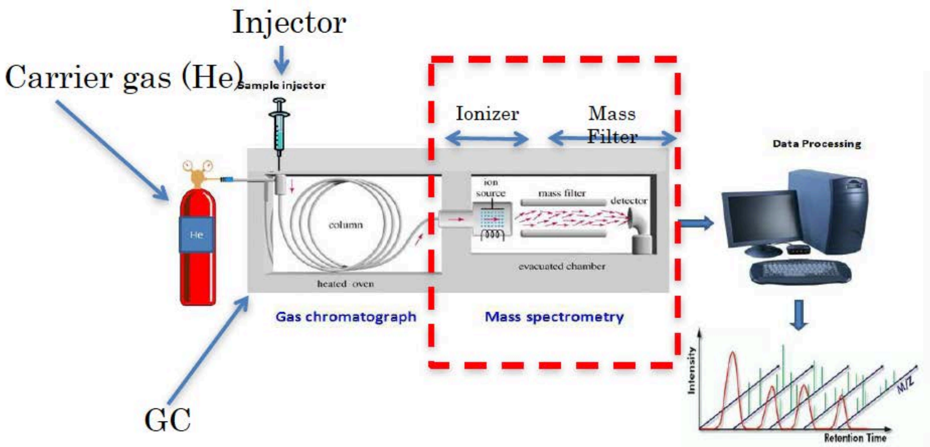
Total fatty acid analysis by GC-MS

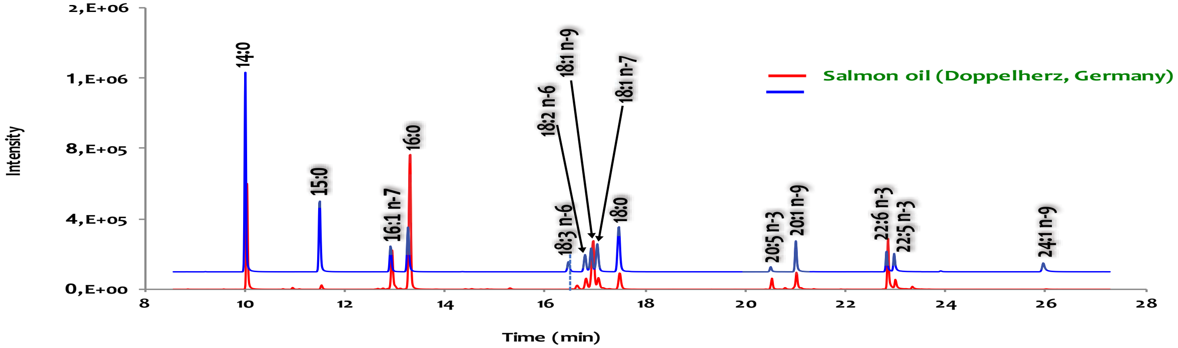


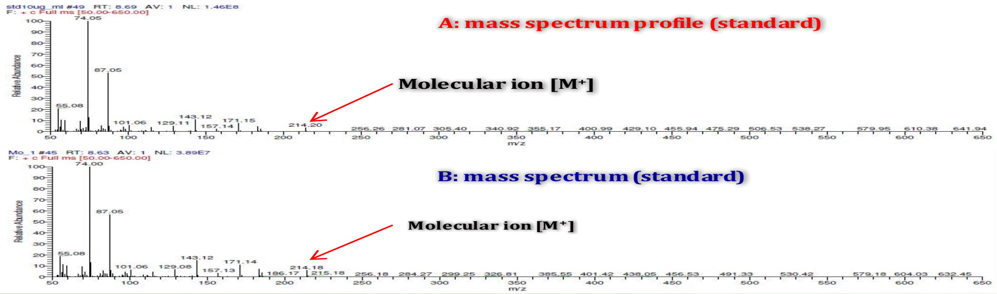
TAG

FAMEs

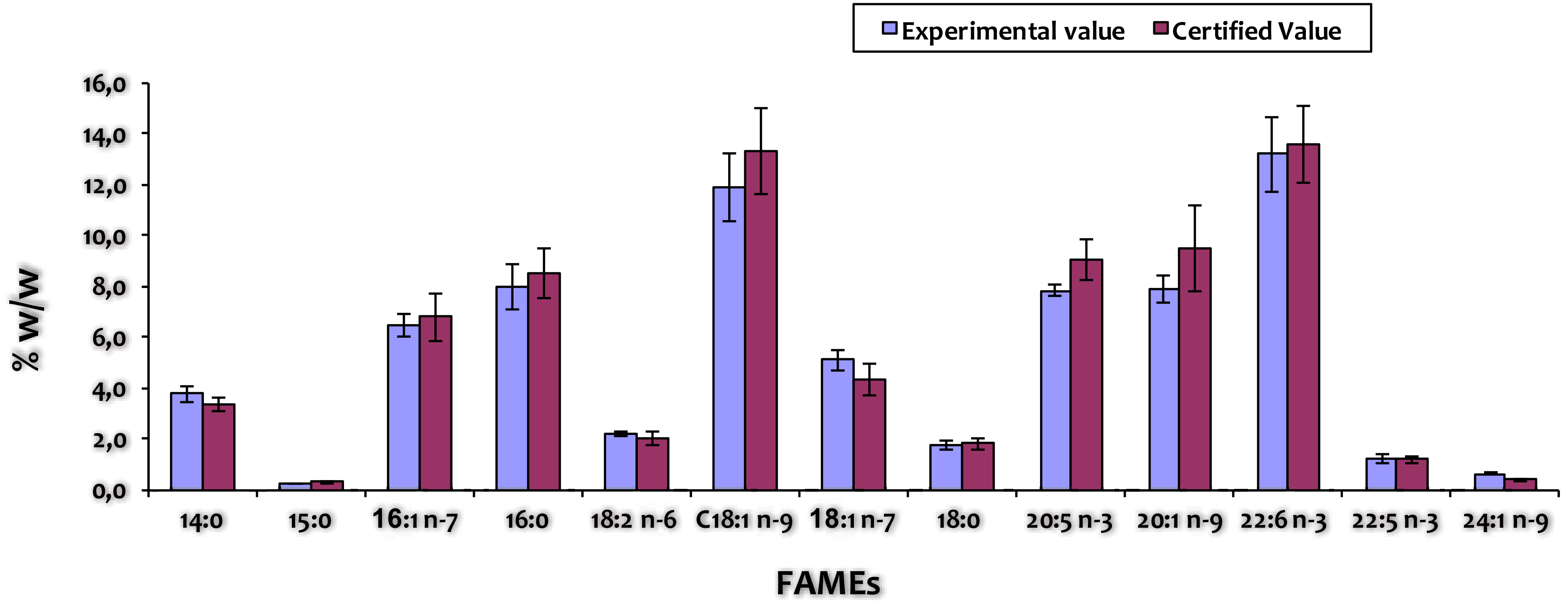
Gylcerol







Test the method



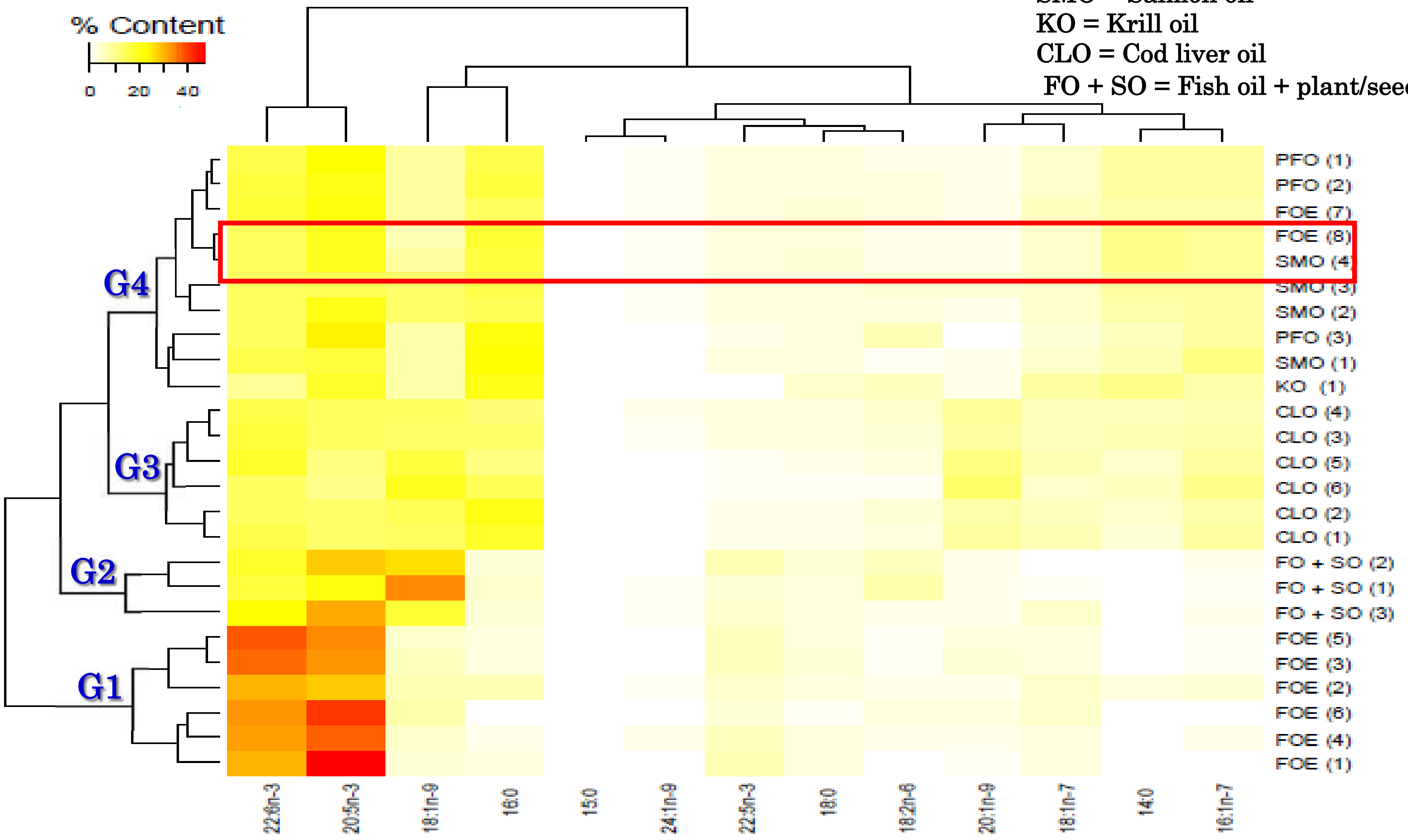
N=3

SRM from National Institute of Standards & Technology, USA

Siriraj Metabolomics & Phenomics Center

Hierarchical clustering algorithm of fatty acid profiles

PFO = Pure fish oil
FOE = Fish oil with an enrichment process
SMO = Salmon oil
KO = Krill oil
CLO = Cod liver oil
FO + SO = Fish oil + plant/seed oil



Example 2

ARTICLE

doi:10.1038/nature09922

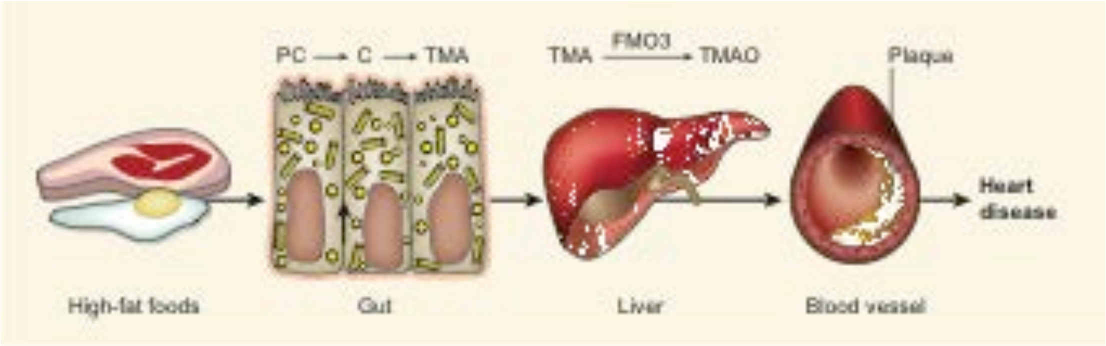
Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease

Zeneng Wang[1,2], Elizabeth Klipfell[1,2], Brian J. Bennett[3], Robert Koeth[1], Bruce S. Levison[1,2], Brandon DuGar[1], Ariel E. Feldstein[1,2], Earl B. Britt[1,2], Xiaoming Fu[1,2], Yoon-Mi Chung[1,2], Yuping Wu[4], Phil Schauer[5], Jonathan D. Smith[1,6], Hooman Allayee[7], W. H. Wilson Tang[1,2,6], Joseph A. DiDonato[1,2], Aldons J. Lusis[3] & Stanley L. Hazen[1,2,6]

Trimethylamine N-oxide (TMAO) and atherosclerosis

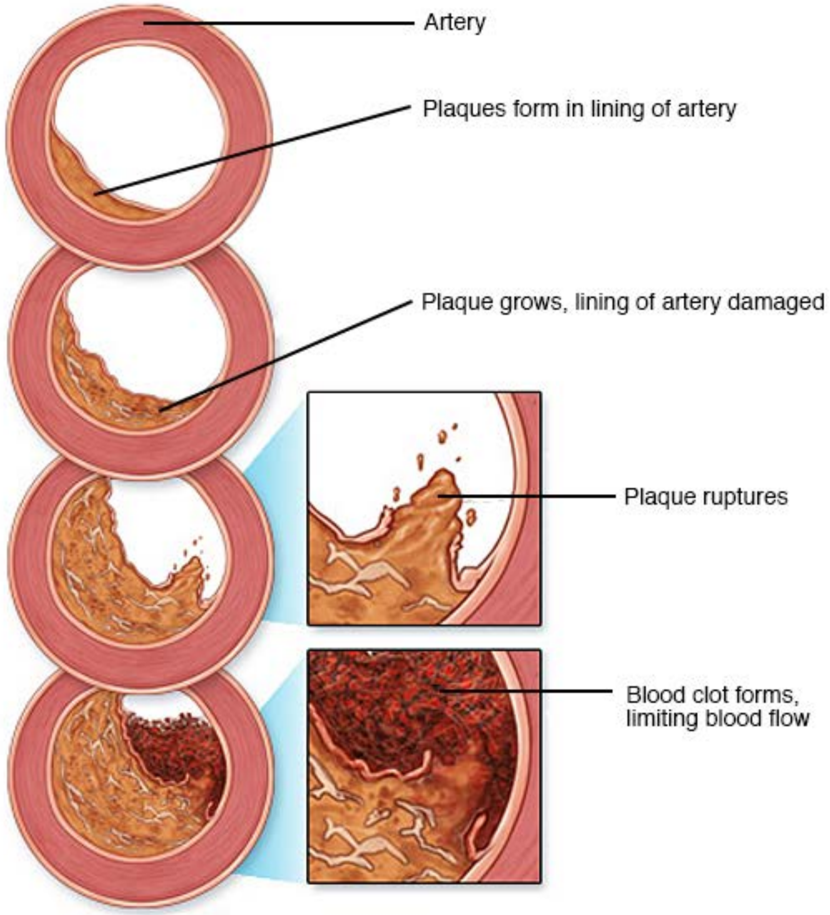
Stanley L. Hazen

Cleveland Clinic Main Campus

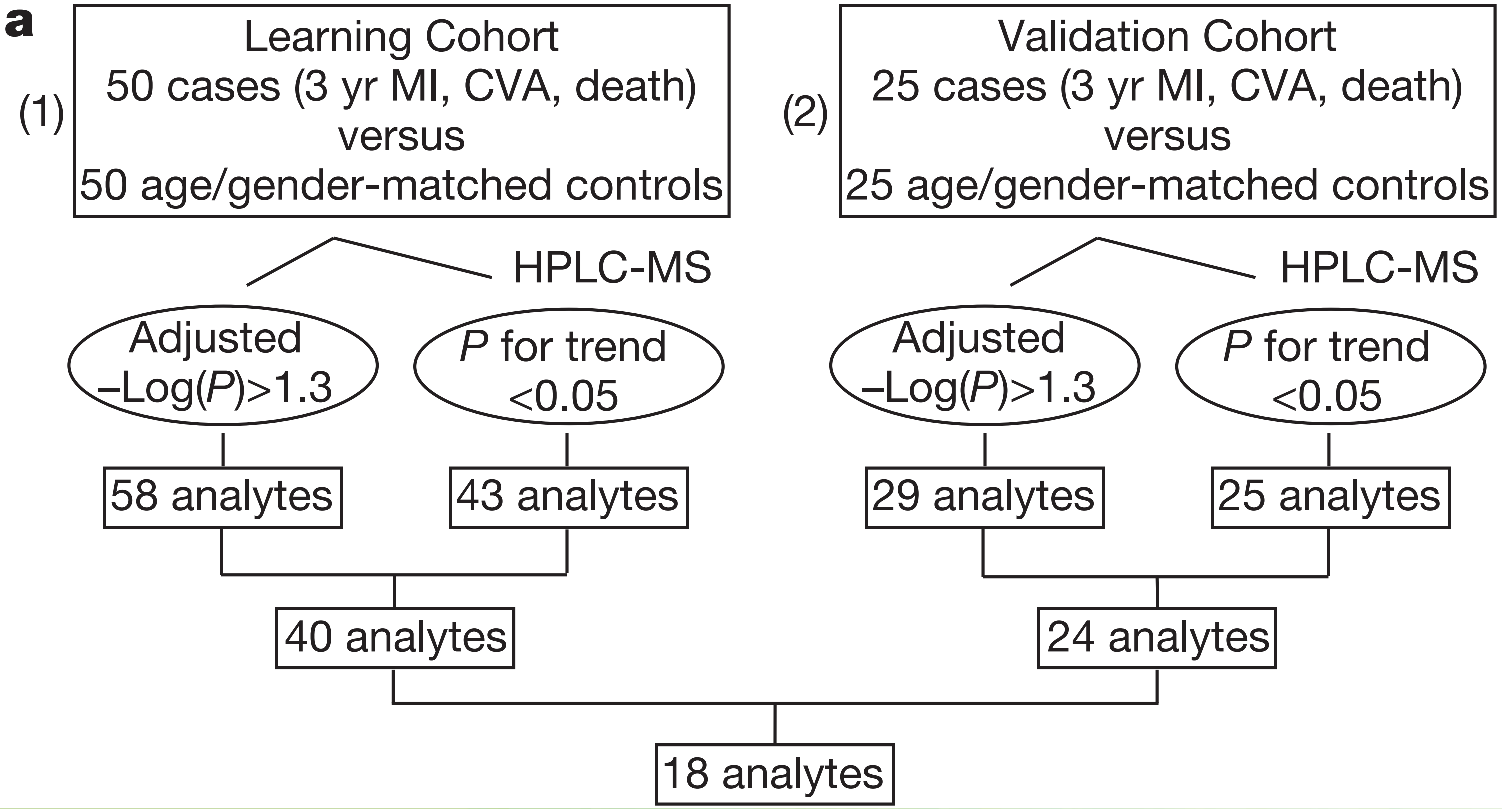


Atherosclerosis

A disease of the arteries characterized by the deposition of fatty material on their inner walls- Normally reattributed from bad gene and high cholesterol

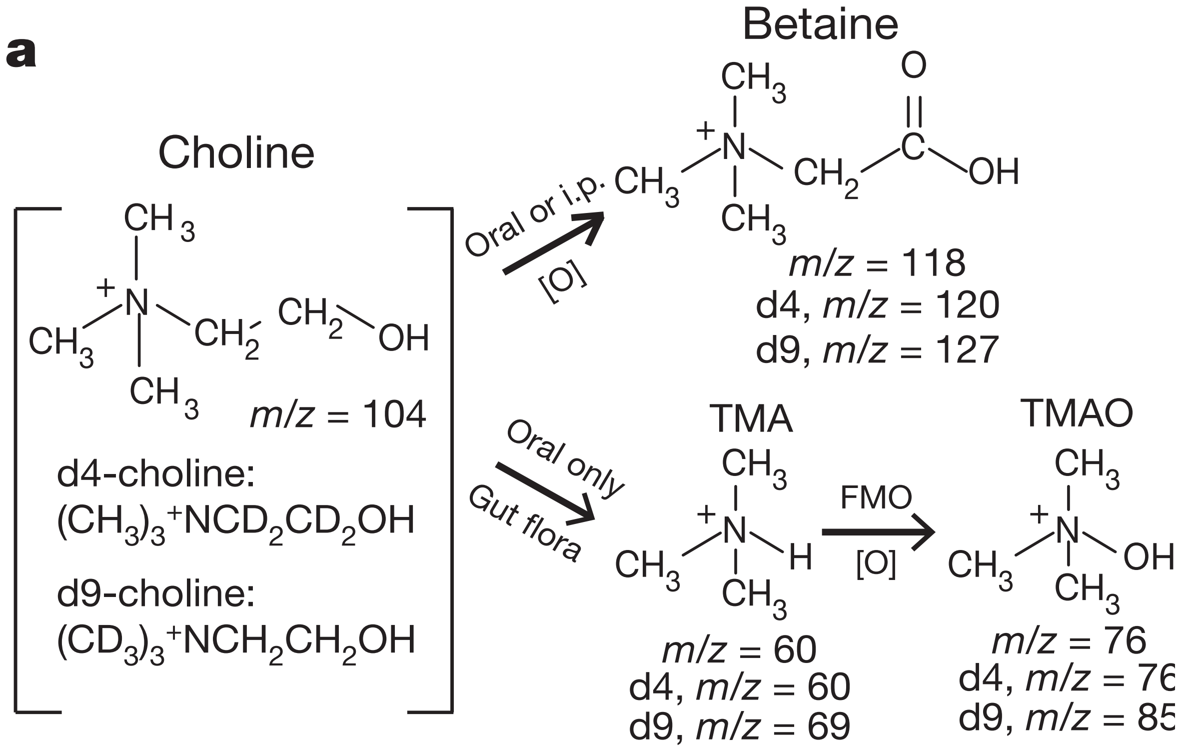


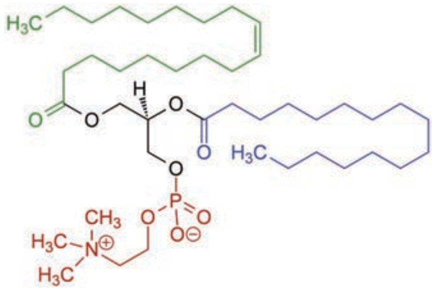
1. Discover metabolite biomarkers in plasma to predict CVD
2. Learning cohort:
   (A) stable patients and subsequently experienced heart attack (myocardial infarction), stroke or death
   (B) A group of age- and gender-matched subjects who did not
3. LC-MS provided <2000 features → 40 metabolites identified as biomarkers
4. An independent cohort identified 18 were identified in both cohorts
5. *m/z* 76, 104 and 118 → top candidates ($P < 0.001$)
6. Structure identification



What are *m/z* 76, 104 and 118 ?

- NMR, LC-MS/MS and GC-MS
- m/z 76 = TMAO (***Trimethylamine* N-*oxide)***



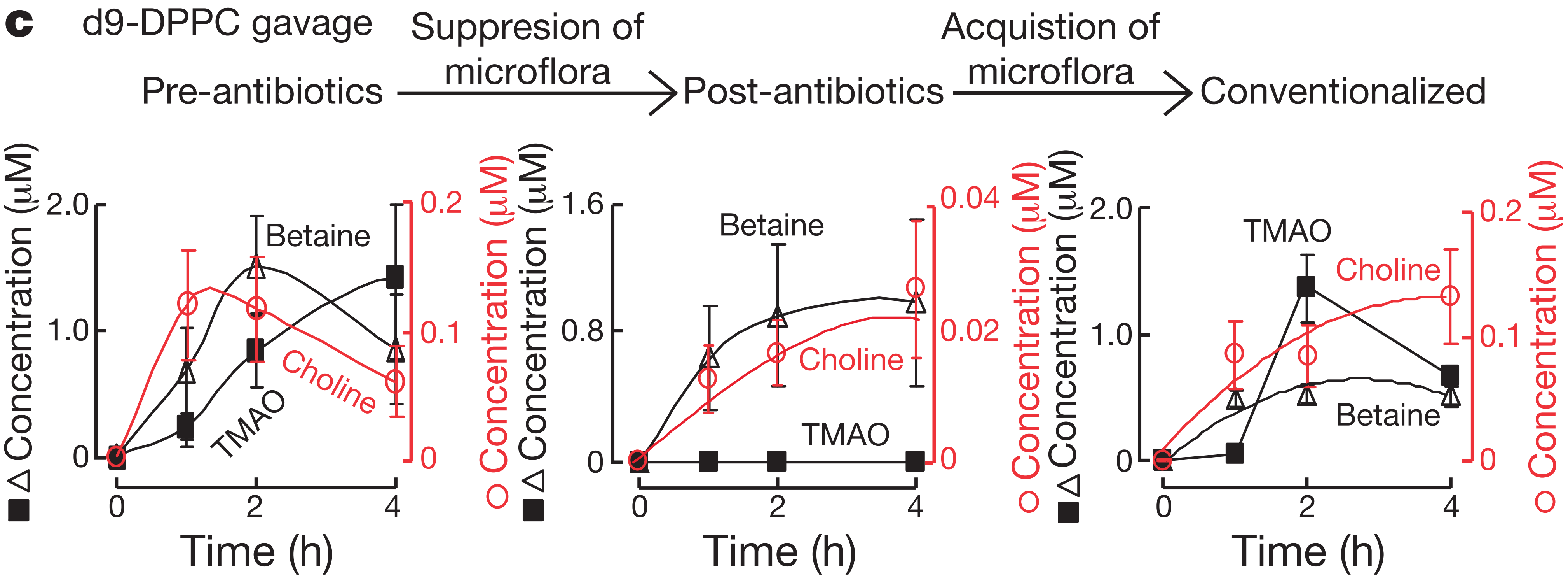


phosphatidylcholine

B

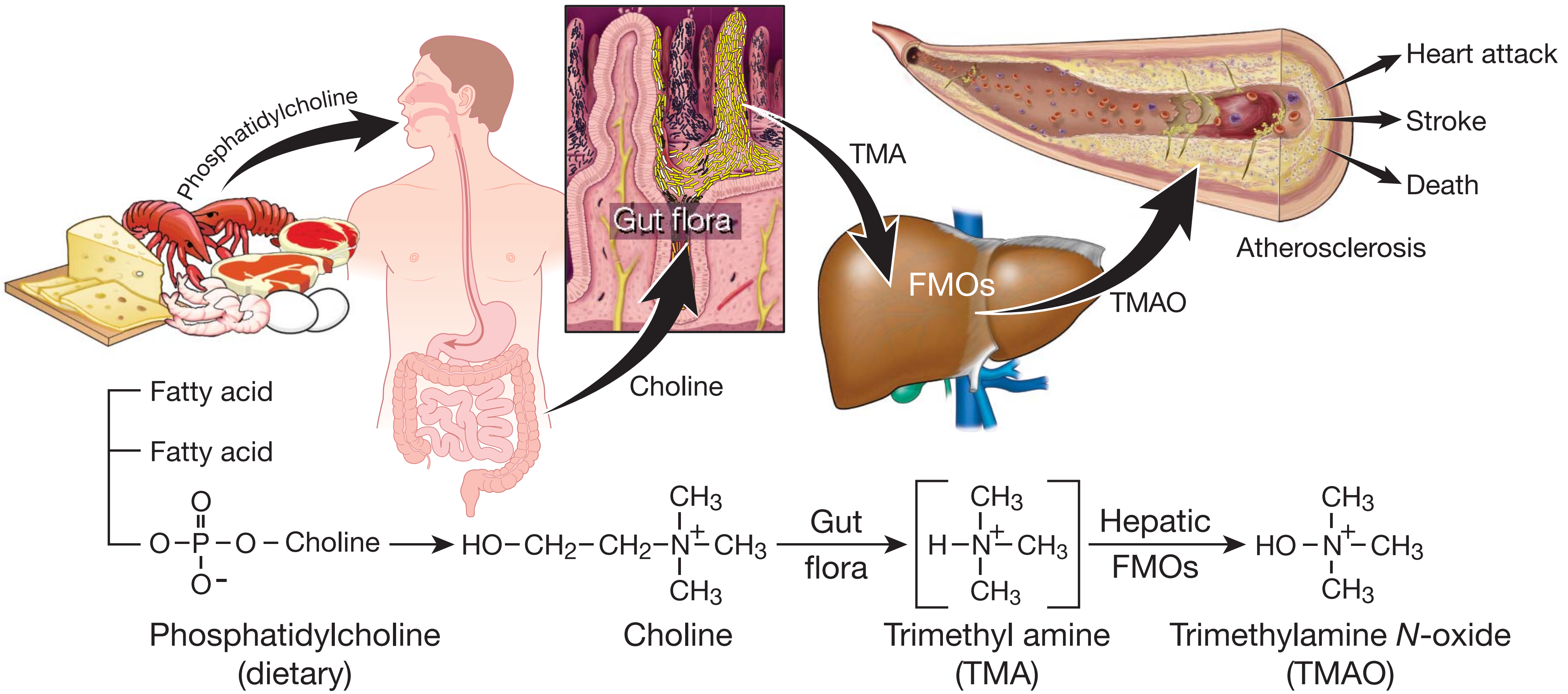
Pre-treatment of antibiotics of
The gut micro flora

Fed with egg-yolk PC



d9-TMAO production after oral d9-DPPC administration in mice, following suppression of gut flora with antibiotics (3 weeks), and then following placement (4 weeks) into conventional cages with non-sterile mice (‘conventionalized’). Data are presented as mean 6 standard error (s.e.) from four independent replicates.

Gut-flora-dependent metabolism of dietary PC and atherosclerosis



Conclusions

1. Introduction to Metabolomics

- Metabolomics ?
- Relation to other –omics → systems biology

2. Metabolomics technologies

- Wet metabolomics
- Dry metabolomics

3. Metabolomics in precision medicine